STUDIES IN PLANT LUMINESCENCE

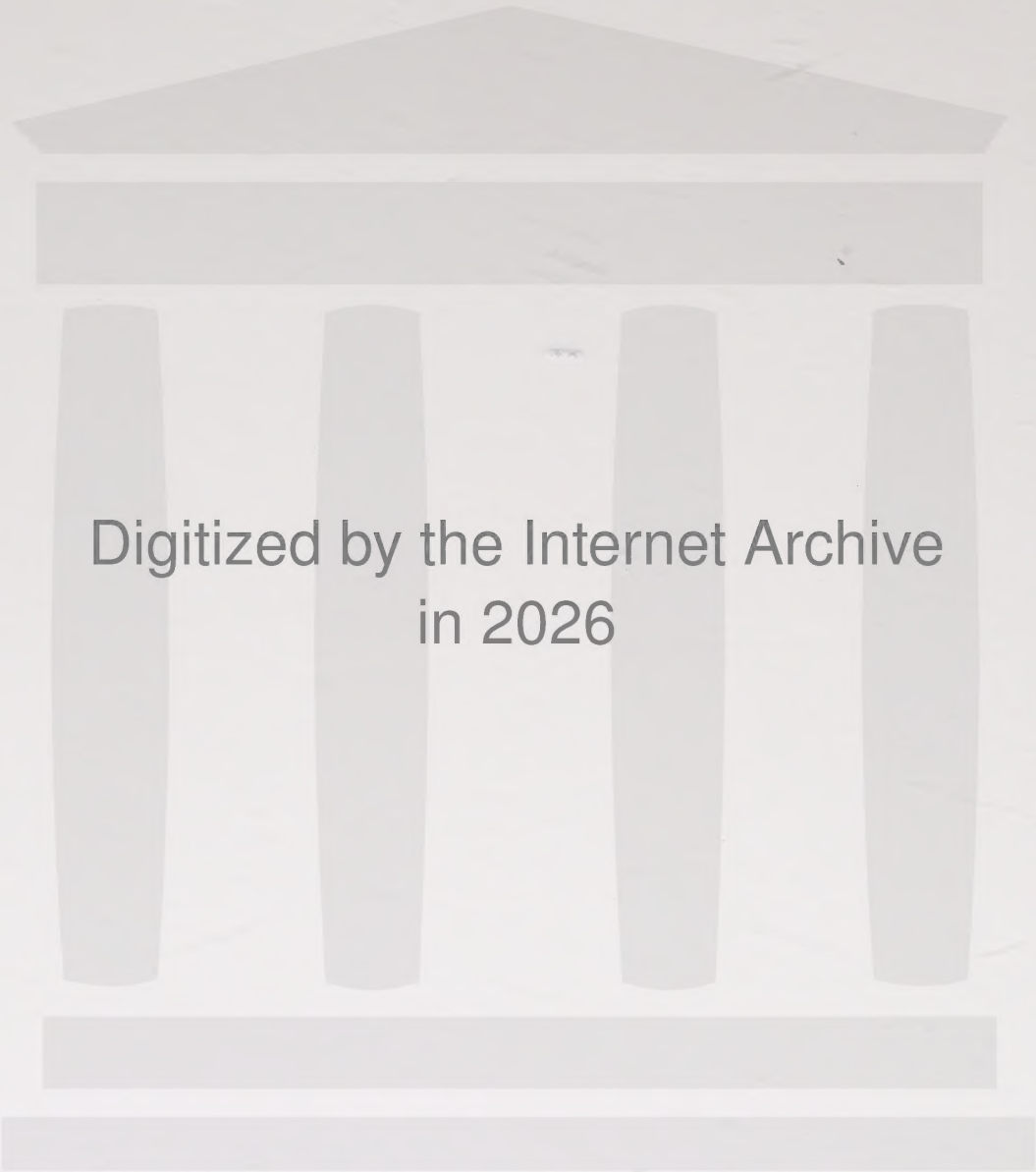
Alcohol-induced luminescence; luminescence in lichens in relation to relative humidity and fluorescence

BJÖRN SIGFRIDSSON

DEPARTMENT OF PLANT PHYSIOLOGY

UNIVERSITY OF LUND, SWEDEN

LUND 1980


Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41553422_0011

Organisation
LUND UNIVERSITY
Department of Plant Physiology
Box 7007
S-220 07 LUND 7
Sweden

Document name
DOCTORAL DISSERTATION

Date of issue
1980-06-02

CODEN:
LUNBDS/(NBFB-1011)/1-84/(1980)

Author(s)
Björn Sigfridsson

Sponsoring organisation
-

Title and subtitle
STUDIES IN PLANT LUMINESCENCE
Alcohol-induced luminescence; luminescence in lichens in relation to relative humidity and fluorescence

Abstract

During photosynthesis carbon dioxide and water are converted into carbohydrate with the aid of light energy, captured by the photosynthetic pigments. However, part of the light captured is re-emitted by the pigments as luminescence. Luminescence measurements provide information about the photosynthetic apparatus and could be useful in evaluating photosynthetic capacity.

PROBLEM: The aim of the research presented here was to investigate whether luminescence could be used to evaluate photosynthetic capacity. Two types of luminescence have been investigated, i.e. A) Alcohol-induced luminescence in Elodea leaves and spinach chloroplasts and B) Light-induced luminescence in lichens under varying humidities. An attempt has also been made to elucidate the mechanism behind these types of luminescence.

METHODS: A) Alcohol-induced luminescence: Leaves from Elodea densa Casp. in water or isolated spinach chloroplasts in 0.35 M NaCl and 20 mM Tris-HCl, pH=8.0, were placed in front of a photomultiplier. After 20 min in darkness (when the light-induced luminescence had reached a negligible level), alcohol was added.

B) Luminescence in lichens under varying humidities: Cladonia impexa Harm. and Collema flaccidium Arch. Ach. were equilibrated with different humidities (regulated by required concentrations of sulfuric acid at the bottom of the cuvettes). The equilibration time was 24 h, the irradiance 9 $W \cdot m^{-2}$, the photoperiod 18 h and the temperature 25°C.

A4 A5

Key words

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN

Recipient's notes

Number of pages
84 pp

Price

Security classification

Distribution by (name and address)
Björn Sigfridsson, Department of Plant Physiology, Box 7007, S-220 07 LUND, Sweden

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature Björn Sigfridsson Date 80-04-17

DOKUMENTDATABLAD enl SIS 61 41 21

STUDIES IN PLANT LUMINESCENCE

Alcohol-induced luminescence; luminescence in lichens in relation to relative humidity and fluorescence

By

Björn Sigfridsson
Fil mag, Mlm

Doctoral dissertation
by due permission of the Faculty of Science
of the University of Lund to be publicly
defended at the Department of Plant Physiology
on June 2nd, 1980, at 10 a.m., for the degree
of Doctor of Philosophy.

CODEN: LUNBDS/(NBFB-1011)/1-84/(1980)

UNIV.
LEIDEN
BIBL.

INTRODUCTION

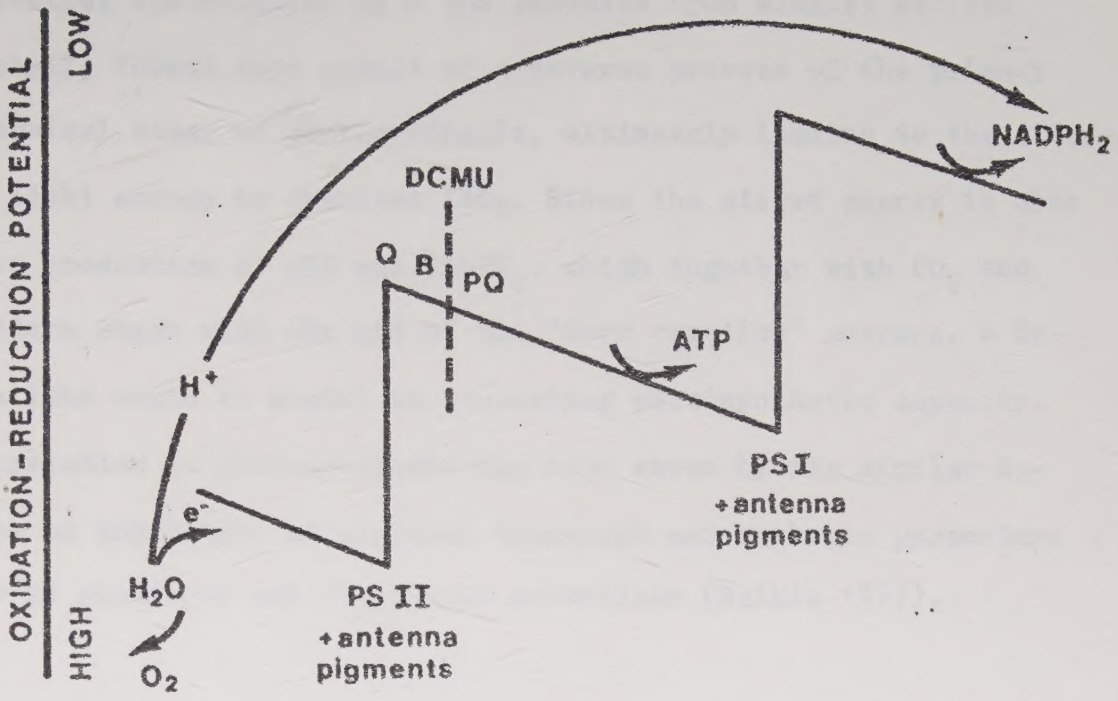
During photosynthesis carbon dioxide and water are converted into carbohydrate with the aid of light energy. This reaction can be divided into two parts. A "light reaction" producing the energy-rich compound ATP and the strong reductant $NADPH_2$ with the aid of light, and a series of enzymatic reactions (often collectively referred to as the "dark reaction") in which these compounds are used for converting carbon dioxide into carbohydrate.

The light reaction takes place in the thylakoid membranes of the chloroplast. The light is captured by pigment molecules distributed between PS II and PS I. With the aid of light absorbed in PS II electrons are pumped from water to Q. On their way to PS I, via the electron carriers B (Bouges-Bocquet 1973) and PQ part of their energy is transferred to ATP. From the energy captured in PS I, the electrons are given a still lower oxidation-reduction potential which can be used for the formation of the strong reductant $NADPH_2$ together with the protons set free when water is split. The oxygen formed is given off.

Only one or two pigment molecules are directly involved in the electron transport chain in each photosystem, making up the reaction centra. Most of the other pigments function as antenna pigments. This means that they serve as light absorbers, transferring their energy to the reaction centra. The antenna pigments are of different kinds in the

---

ABBREVIATIONS: ATP, adenosine triphosphate; B, an electron carrier between PQ and Q; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F, fluorescence; L, luminescence; LH, light-harvesting; $NADPH_2$, reduced nicotinamide adenine dinucleotide phosphate; PS, photosystem; PQ, plastoquinone; Q, the primary electron acceptor of PS II; RH, relative humidity; SDS, sodium dodecyl sulfate.



Schematic representation of photoinduced electron transport in photosynthesis.

different plant divisions. In higher plants and green alga, they are chlorophyll a and b and the carotenoids. In the blue-green algae and red-algae they are phycoerythrin, phycocyanin and allophycocyanin and small amounts of carotenoids and chlorophyll a.

Not all of the carotenoid molecules act as antenna pigments. When plants are exposed to light during which only part of the absorbed light is used for photosynthesis, the excess energy captured may be transferred to oxygen, forming singlet oxygen. This could be harmful since singlet oxygen is very reactive and may cause photooxidation. However, such damage does not seem to occur. According to Krinsky (1971 one way for plants to avoid photooxidation is through the action of carotenoids.

Plants show a long-lived light emission, delayed F or L, after exposure to light (Strehler and Arnold 1951). The light emitted has the same spectral distribution as F and emanates from singlet excited chlorophyll, formed as a result of a reverse process of the primary photochemical stage of photosynthesis, ultimately leading to the storage of the light energy in chemical form. Since the stored energy is also used for production of ATP and $NADPH_2$, which together with CO_2 and H_2O produce sugar with the aid of the "dark reaction" enzymes, L determinations would be useful in evaluating photosynthetic capacity. Its correlation to photosynthesis has been shown by its similar dependence on inhibitors of electron transport and membrane parameters such as pH gradients and electrical potentials (Malkin 1977).

The spontaneous decay of L after light treatment can be modified by chemical or physical treatments, such as salts, organic acids, heating, addition of solvents, changes in external electrical fields and addition of oxidizing and reducing substances. Therefore, L activated by the above treatments has been described as different types of L. However, they all require pre-illumination.

After the spontaneous light-induced L has ceased it is possible to induce L by adding DCMU. This is due to DCMU causing a negative shift of the oxidation-reduction potential of B, lowering it below that of Q. Thus Q is reduced and when its electrons recombine with the positive charges on the oxidative side of PS II, excited chlorophyll is formed. The basis for this type of L is derived from the fact that B will keep the negative charges, even after the spontaneous decay of L. It has not been determined whether these charges are remains of previous electron flow or if they come from some other metabolic process (Etienne and Lavorel 1975).

However, L should not be possible if energy is only supplied as light energy. Any other addition of energy causing the formation of excited chlorophyll should give L. This was shown by inducing L when ATP was added (Schreiber et al. 1977).

L is thus a complex phenomenon, which, although certainly correlated to photosynthesis, can be supposed to reflect this process differently according to different prevailing conditions.

The aim of the research presented here was to investigate whether L could be used to evaluate photosynthetic capacity. To illuminate this problem two types of L have been investigated, namely L induced by alcohols in Elodea and isolated chloroplasts, and L induced by light in lichens under varying humidities. An attempt has also been made to elucidate the mechanism behind alcohol-induced L and the causes of changes with changing humidity in lichen photosynthesis.

This thesis is based on the following papers.

I. Björn, L.O. & Sigfridsson, B. 1971. Luminescence induced in intact leaves and isolated chloroplasts by alcohols and other substances. - Physiol. Plant. 25:308-315.

II. Sigfridsson, B. 1978. Factors affecting ethanol-induced luminescence in dark-treated chloroplasts. - Physiol. Plant. 44:256-260.

III. Sigfridsson, B. 1980. Some effects of humidity on the light reaction of photosynthesis in the lichen Cladonia impexa and Collema flaccidium. - Physiol. Plant. In press.

IV. Sigfridsson, B. & Öquist, G. 1980. Preferential distribution of excitation energy into photosystem I of desiccated samples of the lichen Cladonia impexa and the isolated lichen-alga Trebouxia pyriformis. - Physiol. Plant. In press.

MATERIALS AND METHODS

Alcohol-induced luminescence:

L from leaves of Elodea densa Casp. was determined in a plexiglass cuvette filled with distilled water and placed in front of a photomultiplier (Hamamatsu R-374). The signal was amplified and recorded. After 20 min in darkness (when the light-induced L had reached a negligible level), the alcohol was added to the water. The material was handled in dim green light and no measurable L was induced. Eleven different alcohols with up to ten carbon atoms were tested.

L measurements were also carried out with chloroplasts isolated from leaves of spinach (Spinacea oleracea L. cv. Viking, Weibulls, Landskrona, Sweden) grown in soil in a greenhouse. The isolated chloroplasts were resuspended in 0.35 M NaCl and 20 mM Tris-HCl, pH 8.0. After being placed in darkness for 20 min in a test-tube in front of the photomultiplier, light-induced L had reached a negligible level. Ethanol was then added with a syringe. Addition of test substances took place either before or after the addition of ethanol. The sample was continuously aerated.

Luminescence and room temperature fluorescence from lichens:

A piece of Cladonia impexa Harm. about 3×4×5 cm was placed in an air-tight plexiglass cuvette of about 500 ml volume. Humidity was regulated with 80 ml sulfuric acid of required concentration (Stokes and Robinson 1949) at the bottom of the cuvette. L and F were induced by blue light. L was measured 3 ms after excitation.

Fluorescence emission spectra from Collema flaccidium Ach. Ach. were measured in an Aminco-Bowman Spectrofluorometer in 1×1 cm glass cuvettes. A small piece of the lichen, about 5×5 mm was placed at an angle of 45° to the excitation light. Humidity was regulated by 0.5 ml sulfuric

acid of required concentration at the bottom of the cuvette.

Both lichens were subjected to an irradiance of 9 $W \cdot m^{-2}$, a photoperiod of 18 h and a temperature of 25°C during 24 h of equilibration with the prevailing humidity in the cuvettes.

Low temperature fluorescence measurements:

Cladonia impexa Harm., kept in air-tight plexiglass cuvettes, was exposed to a photoperiod of 17 h, a temperature of 25°C in the light and 15°C in the dark and an irradiance of 10 $W \cdot m^{-2}$. Humidity was regulated by sulfuric acid of required concentration. The equilibration time was 24 h. Emission spectra were measured on a fluorimeter constructed at the Department of Plant Physiology at the University of Umeå (Öquist 1974). The sample was cooled by putting it into direct contact with liquid nitrogen in the Dewar vessel, immediately after removal from the plexiglass cuvette.

Isolated lichen alga Trebouxia pyriformis Archibald and the non-lichen alga Scenedesmus obliquus, strain D_3 (obtained from N.I. Bishop), were soaked onto filter paper, and placed in direct contact with liquid nitrogen in the Dewar vessel.

Scenedesmus was grown heterotrophically in continuous light of 30 $W \cdot m^{-2}$ and a temperature of 27°C. Trebouxia was grown in continuous light of 2 $W \cdot m^{-2}$ and a temperature of 25°C in a basic mineral medium supplemented by glucose and peptone under aseptic conditions.

ALCOHOL-INDUCED LUMINESCENCE

Dark-treated Elodea leaves emit light when treated with alcohol. Compared to the light-induced L, the intensity of the emitted light is low, Its duration, however, is very long, up to 13 h for heptanol. The concentration needed for L is different for different alcohols. High molecular alcohols need a lower concentration than low molecular ones, and branched long-chain alcohols are less effective than their unbranched isomers. The kinetics of light emission for one and the same alcohol is concentration dependent. For isobutanol at a low concentration (3.4 % by volume), light emission increases slowly and the maximum is not reached within 30 min. If the concentration is increased, the maximum is reached faster. Concomitantly the peak is increased; nearly doubled when the concentration is increased from 4.8 to 9.1 % by volume.

Isolated chloroplasts need a higher (about 3 times for ethanol) alcohol concentration for light emission than do intact plants, probably due to a concentration effect in the intact plant. The high ethanol concentration needed to induce L from chloroplasts causes chlorophyll solubilization. This means that normal physiological reactions such as electron transport, establishment of a hydrogen gradient and photophosphorylation have ceased (Masamoto et al. 1978). In accordance with this, L induced by ethanol is not quenched by NH_2OH and DCMU, either separately or combined, in contrast to the spontaneously decaying L induced by light treatment.

Ethanol induces L after the spontaneously light-induced L has reached a negligible level. This means, that the two types of L depend on different energy pools. However, it cannot be ruled out that ethanol-

induced L depends on a previous illumination. If so, the activation energies for the two pools must be different. The energy of the light emission is not due to the electrons stored in B after a preillumination, since these are kept only for 40 min (after that time DCMU will not induce L from dark-treated chloroplasts) and ethanol can induce L even after this time. Ethanol-induced L is further independent of oxygen concentration down to 20 % of that in air. This is in contrast to the L found in intact systems i.e. far-red induced afterglow (Björn 1971). L induced by ethanol thus shows many differences as compared to L from intact chloroplast systems.

Krasnovskii Jr. and Litvin (1974) have studied L from chlorophyll in organic solvents. According to them, L is due to a disintegration of chlorophyll-peroxides, created by a reaction between chlorophyll and singlet oxygen. In their system as well as in the one studied here, the light emission is quenched by copper ions, known to destroy peroxides. Even if the light emission in the present system is due to a breakdown of peroxides, they are created in another way than in the experiments of Krasnovskii Jr. and Litvin 1974, since NaN_3, a quencher of singlet oxygen, does not quench the light emission. However, the light emission emanates from a singlet excited state, shown by the susceptibility to m-dinitrophenol. A possible source for the emitted energy could be that liberated when the lipid-aqueous interfacial area decreases upon addition of alcohol.

Although ethanol induces strong structural disintegration a structural requirement for the L is likely. This is in agreement with the fact that ethanol-induced L cannot be obtained if the chloroplasts are boile or treated with SDS in contrast to when they are treated with Triton

X-100 or digitonin (milder detergents). The fact that it is pH-dependent and quenched by acetone that has a stronger denaturating effect on proteins than does ethanol (Huang et al. 1971) also points to a structural dependence. It is also sensitive to oxidizing and reducing substances, but this does not necessearily point to a structural dependence.

INFLUENCE OF HUMIDITY ON LUMINESCENCE AND FLUORESCENCE IN LICHENS

Photosynthesis proceeds in lichens only at high RH i.e. 80 % or more (Bertsch 1966). Above 80 % RH L increases with increasing RH. Between 50 and 80 %, however, L is constant. The increase above 80 % is in agreement with the increased photosynthesis. L is thus composed of two components, one related and one that is unrelated to photosynthesis.

The response of L to humidity is rapid. Therefore, a regulation system based on degrading and rebuilding of larger components in the photosynthetic apparatus seems unlikely. Instead some kind of switch, turning photosynthesis on and off according to the prevailing humidity has been proposed. The reason for this is outlined below, as well as a proposal for its function.

Inhibition of photosynthesis can be achieved either by some step in the "dark reaction" or in the "light reaction". At low RH electron transport is stopped, (shown by DCMU having no effect on F and L at low RH) no $NADPH_2$ can be formed. Further, the electron storing capacity in the electron transport chain is reduced as RH is lowered (shown by F induction measurements). Finally, interference with ATP production

at 95 % RH and lower is presumed, since the kinetics of far-red-induced L (a type of L presumably dependent on photophosphorylation (Bertsch and Azzi 1965)) is sensitive to RH.

As the RH drops, chlorophyll F sensitized by antenna pigments is decreased. In Collema, containing a blue-green alga, excited phycoerythrin and phycocyanin are deexcited by F via allophycocyanin at low RH. This is not due to a substantially decreased chlorophyll a F yield, since F, excited by direct chlorophyll a excitation, is rather independent of RH. At high RH, chlorophyll a F is sensitized either by direct chlorophyll a excitation or by excitation of phycocyanin and phycoerythrin. When RH drops, there is in Cladonia a decreased yield of F sensitized by chlorophyll b as compared to the yield of F sensitized by chlorophyll a.

With decreasing L, F decreases as RH is lowered. This is puzzling because inhibited photosynthesis (here observed as inhibited L) normally results in diminished F since both processes benefit from the same pool of energy. Thus a decrease in one of them would result in an increase of the other. However, photosynthesis stopped by DCMU at high RH results in increased F. Thus, low RH not only causes a break in the electron transport between the two PS, but also impairs energy transfer routes.

According to the Z-scheme (Hill and Bendall 1960) the two photosystems operate in series. Since their absorption spectra differ, maximum quantum efficiency of photosynthesis should be obtained only at specific wavelengths. However, this is not the case. The quantum efficiency of photosynthesis for green algae is essentially independent of wavelengths from 570 to 685 nm (Emerson and Lewis 1943). This has been explained as

being due to a mechanism (spill-over, Myers and Graham 1963) which redistributes part of the captured energy between the two photosystems so that overexcitation of PS II compared to PS I results in energy transfer from PS II to PS I. Thus, increased energy transfer from PS II to PS I could explain the simultaneous reduction in F and L when RH drops, since room temperature F reported so far emanates from PS II.

Three different chlorophyll-protein complexes have been isolated from chloroplasts. Two of these contain the respective reaction centra of the two photosystems plus their associated pigments (mainly chlorophyll a and carotenoids). The third one, i.e. the LH complex, contains mainly chlorophyll a and b (in higher plants and green algae) and has no reaction centra. Its main function is to increase light absorption. The absorbed energy is transferred to the two photosystems, preferentially into PS II. At low temperature three emission bands appear in the F spectra. These have been assigned to the two photosystems (with their associated pigment-proteins) and the LH complex, respectively. As the F emitted reflects the energy content in the complexes, F sensitized by chlorophyll b will reveal energy transfer into the two photosystems. Low temperature F measurements on Cladonia revealed a spill-over mechanism. It is light-dark sensitive, as found in algae (Murata 1968) and sensitive to RH. At low RH, when photosynthesis is inhibited, captured energy is preferentially distributed into PS I.

Excess energy in PS II has been shown to create a strong oxidant on the oxidative side of PS II, causing carotenoid oxidation (Fujita and Suzuki 1973). The danger of having a persisting PS II activity when photosynthesis is inhibited has been shown by Fork (1973) and by Wiltens et al. (1978). Algae with a retained PS II activity become more

damaged when exposed to light when photosynthesis is inhibited than those which are able to cut off PS II activity completely. This has also been shown in this investigation as Scenedesmus does not show the same F emission spectrum when rewetted after drying (compared to before drying) in contrast to Trebouxia. Also in contrast to Trebouxia, Scenedesmus partly retains PS II activity when dry. Besides causing the formation of a strong oxidant on the oxidative side of PS II when photosynthesis is inhibited, excited chlorophyll may transfer energy onto oxygen, giving singlet oxygen which may cause photo-oxidation. According to Krinsky (1971), one way plants avoid this is through the protective role of carotenoids (including β-carotene). The β-carotene found in PS I (Junge et al. 1977) could thus function as a trap for excess excitation energy (Öquist et al. 1980).

Thus lichens have solved the problem of being exposed to light when photosynthesis is inhibited by transfer of the excitation energy into PS I. The spill-over mechanism has been utilized for this. The ability to handle this problem can be supposed to be especially important in lichens, since they, in contrast to higher plants, do not have transpiration protection mechanisms.

Spill-over in isolated chloroplasts can be regulated by the ion concentration in the suspension medium (Murata 1969). Since a variety of inorganic metal ions possess this ability (Murata et al. 1969), this has been interpreted to be due to their influence on protein configuration. These conformational changes would in turn influence distance and orientation between pigments, causing changes in energy transfer (Förster 1948). Even a change of a few chlorophyll molecules would be enough to cause changes in spill-over (Seely 1973). Other

treatments, causing changes in protein configuration, could thus be supposed to regulate energy transfer. Changes in protein configuration in turn could be brought about by changes in water potential. This regulation mechanism would be very suitable for lichens, since they lack transpiration protection and therefore easily equilibrate their water potential with that of the surrounding air.

The protection against lethal photo-oxidation when photosynthesis is inhibited could not be due to the amount of β-carotene in the lichen algae, since it is about the same as in other alga. Instead, the protection mechanism would be the effective preferential transfer of absorbed energy into PS I at low RH values. This also results in inhibited photosynthesis, and has been proposed as the switch that turns photosynthesis on or off in accordance with humidity.

CONCLUSION

L can be used both for measuring photosynthetic capacity and in the investigation of the photosynthetic mechanism.

Before L can be used for measuring photosynthetic capacity, the relation between L and photosynthetic capacity must be checked for different types of L and different plant materials.

A L type not related to photosynthesis is L induced by alcohols, although it is due to deexcitation of singlet excited chlorophyll and is dependent on chloroplast structure.

Even in intact systems, L cannot uncritically be assumed to be proportional to photosynthesis. In lichens, L shows two components, one related to photosynthesis and one that is not related.

When elucidating the photosynthetic system, L is a valuable parameter in accounting for energy distribution within the intact photosynthetic apparatus. In this work advantage of this was taken. The result was a demonstration of spill-over as a mechanism for adaptation of the photosynthetic apparatus in lichens to the environmental air humidity.

ACKNOWLEDGEMENTS

I am indebted to my supervisor, Professor Lars Olof Björn, for valuable suggestions. I am very grateful to Dr. Gunnar Öquist of the Department of Plant Physiology at the University of Umeå for the stimulating cooperation which resulted in paper IV. Thanks are also due to Mr. Ingvar Jönsson for cultivation of plant material and to Mrs. Lena Mundt-Larsson for preparation of the figures. I also want to thank all other persons who have stimulated and helped me in this work.

REFERENCES

Bertsch, A. 1966. Ueber den CO_2-Gaswechsel einiger Flechten nach Wasserdampfaufnahme. - Planta 68:157-166.

Björn, L.O. 1971. Far-red induced, long-lived afterglow from photosynthetic cells. Size of afterglow unit and paths of energy accumulation and dissipation. - Photochem. Photobiol. 13:5-20.

Bouges-Bocquet, B. 1973. Electron transfer between the two photosystems in spinach chloroplasts. - Biochim. Biophys. Acta 314:250-256.

Emerson, R. & Lewis, C.M. 1943. The dependence of the quantum yield of Chlorella photosynthesis on wave length of light. - Am. J. Bot. 30:165-178.

Etienne, A.L. & Lavorel, J. 1975. Triggered-luminescence in dark adapted Chlorella cells and chloroplasts. - FEBS Lett. 57(3):276-279.

Fork, D.C. & Hiyama, T. 1973. The photochemical reactions of photosynthesis in an alga exposed to extreme conditions. - Carnegie Inst. Yearbook 72:184-188.

Fujita, Y. & Suzuki, R. 1973. Studies on the Hill reaction of membrane fragments of blue-green algae IV. Carotenoid photobleaching induced by photosystem II action. - Plant Cell Physiol. 14:261-273.

Förster, Th. 1948. Zwischenmolekulare Energiewanderung und Fluoreszenz. - Ann. Phys. 2:55-75.

Hill, R. & Bendall, F. 1960. Function of the two cytochromes in chloroplasts. A working hypothesis. - Nature 186:136-137.

Huang, K., Tao. M.C., Boulet, M., Riel, R.R., Julien, J.P. & Brisson, G.J. 1971. A process for the preparation of leaf protein concentrates based on treatment of leaf juices with polar solvents. - J. Inst. Can. Technol. Aliment. 4(3):85-90.

Junge, W., Schaffernicht, H. & Nelson, N. 1977. On the mutual orientation of pigments in photosystem I particles from green plants. - Biochim. Biophys. Acta 462:73-85.

Krasnovskii, A.A.,Jr. & Litvin, F.F. 1974. Long-lived afterglow of photosynthetic pigments in solutions: Photochemiluminescence. - Photochem. Photobiol. 20:133-149.

Krinsky, N.I. 1971. Function. - In Carotenoids (O. Isler, ed.) pp. 669-716. Birkhäuser Verlag, Basel and Stuttgart.

Malkin, S. 1977. Delayed luminescence. - In Encyclopedia of Plant Physiology New Series. Volume 5. Photosynthesis I. (A. Trebst & M.Avron eds.). pp 473-491. Springer-Verlag, Berlin, Heidelberg, New York. ISBN 3-540-07962-9.

Masamoto, K. & Nishimura, M. 1978. Effects of ethanol on the interaction of photosynthetic processes in spinach chloroplasts. - Plant Cell Physiol. 19(8):1543-1552.

Murata, N. 1968. Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in Porphyridium cruentum. - Biochim. Biophys. Acta 172:242-251.

Murata, N. 1969. Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in spinach chloroplasts. - Biochim. Biophys. Acta 189:171-181.

Murata, N., Tashiro, H. & Takamiya, A. 1969. Effects of divalent metal ions on chlorophyll a fluorescence in isolated spinach chloroplasts. - Biochim. Biophys. Acta 197:250-256.

Myers, J. & Graham, J. -R. 1963. Enhancement in Chlorella. - Pl. Physiol. 38:105-116.

Öquist, G. 1974. Iron deficiency in the blue-green alga Anacystis nidulans: Fluorescence and absorption spectra recorded at 77 K. - Physiol. Plant. 31:55-58.

Öquist, G., Samuelson, G. & Bishop, N.I. 1980. On the role of β-carotene in the reaction center chlorophyll a antenna of photosystem I. - Physiol. Plant. In press.

Schreiber, U. & Avron, M. 1977. ATP-induced chlorophyll luminescence in isolated spinach chloroplasts. - FEBS Lett. 82(1):159-162.

Seely, G.R. 1973. Energy transfer in a model of the photosynthetic unit of green plants. - J. Theor. Biol. 40:189-199.

Stokes, R.H. & Robinson, R.A. 1949. Standard solutions for humidity control at 25°C. - Industr. Engng. Chem. 41, p. 2013.

Strehler, B.L. & Arnold, W. 1951. Light production by green plants. - J. Gen. Physiol. 34:809-820.

Wiltens, J., Schreiber, U. & Vidaver, W. 1978. Chlorophyll fluorescence induction: an indicator of photosynthetic activity in marine algae undergoing desiccation. - Can. J. Bot. 56:2787-2794.

Luminescence Induced in Intact Leaves and Isolated Chloroplasts by Alcohols and Other Substances

By

L. O. BJÖRN and BJÖRN SIGFRIDSSON

Department of Plant Physiology, University of Lund, Sölvegatan 35, S-223 62 Lund

(Received May 17, 1971)

Abstract

Elodea leaves and spinach chloroplasts emit red light when treated with alcohols or certain other solvents for chlorophyll. The intensity of the light, the lag phase and the threshold concentration vary considerably between different alcohols.

Light emission from a leaf starts a few seconds to a few minutes after the addition of alcohol to the medium, reaches a maximum after 2–45 minutes (sometimes more) and then continues for many hours. Despite the faintness of the glow, the total number of photons given off from a leaf after addition of alcohol may exceed the number of photons given off from the same sample as long-lived afterglow after saturating irradiation with far-red light. The maximum yield of photons per chlorophyll molecule is a little more than 10^{-5}. The alcohol-induced luminescence is not influenced by a decrease in the oxygen tension to one fifth of the normal.

Electron micrographs of treated leaves reveal that the thylakoid lipids contract to drops at the edges of the grana. Treatment of isolated chloroplasts with ethanol results in light emission when the concentration is high enough to dissolve the chlorophyll. It is estimated that the surface free energy in the thylakoid lipid-aqueous interface, due to ordinary interfacial tension, is large enough to account for the light emission observed when the interfaces contract or disappear.

Introduction

Photosynthetic cells and isolated chloroplasts exhibit a number of different types of luminescence: fluorescence, delayed light emission or afterglow (Strehler and Arnold 1951), luminescence triggered by sudden changes in pH or salt concentration (Mayne and Clayton 1966, Mayne 1968, Miles and Jagendorf 1961, Barber and Kraan 1970) and thermoluminescence (Arnold 1966). This paper adds another phenomenon to the list: luminescence induced by solvents for chlorophyll.

The effect was first observed during an investigation of the influence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on a special type of afterglow (Björn 1971 b) in which ethanol was tried as solvent for a stock solution of the substance.

Materials and Methods

Elodea densa Casp. was obtained commercially. Routinely 100 leaves were used in the sample holder described elsewhere (Björn 1971 b), in which they were immersed in aerated, distilled water to which the substance to be tested was added. Alcohols with 5 or more carbon atoms are not readily soluble in water and were first dissolved in methanol. The solution was then diluted ten times with water to yield an emulsion. Ten percent methanol does not by itself cause any luminescence. In expressions of concentration, "percent" refers to percent by volume.

Spinacea oleracea L. cv. Viking (Weibulls, Landskrona, Sweden) was grown in soil in a greenhouse. Chloroplasts were prepared in a medium containing sodium chloride (0.35 *M*), tris-(hydroxymethyl)-aminomethane ("tris", 20 m*M*) and ascorbic acid (12 m*M*) After sedimentation (approx. 7 minutes at 1000 g) they were resuspended in 0.35 *M* sodium chloride buffered with 20 m*M* "tris" to which hydrochloric acid had been added to pH 8.0.

For the experiments with chloroplasts a cuvette was used in which the back was made of mirror glass. In this way the sensitivity of the apparatus was increased, but since no calibration with this geometry was made, only relative values were obtained.

In an experiment to determine the importance of oxygen for the luminescence of *Elodea* leaves, a cuvette was used that could accomodate the electrode of a Beckman model 777 oxygen analyzer. In this experiment air and nitrogen were alternately bubbled through the liquid in which the leaves were immersed.

The luminescence measurements were made with a

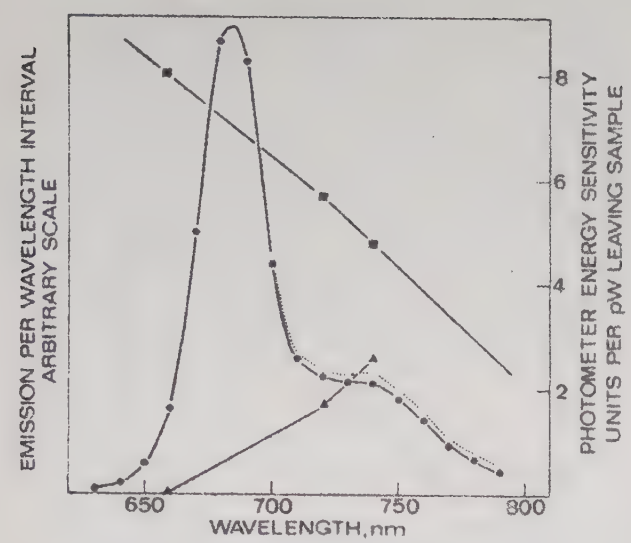


Figure 1. *Emission spectra and photometer sensitivity.* Circles: Fluorescence spectrum of a single leaf (filled circles and solid line show energy spectrum, open circles and dashed line quantum spectrum). Triangles: Energy spectrum of isobutanol-induced luminescence from a cuvette packed with 100 Elodea leaves, as measured through interference filters. Squares and right ordinate: Spectral energy sensitivity of photometer. The abscissa shows wavelength in nm in all cases.

Hamamatsu R 374 photomultiplier. The photocathode was about 17 mm from the sample, and it was calculated that about 1/12 of isotropic radiation from the sample would impinge upon the photocathode. A shutter between sample and photomultiplier made it possible to correct for dark-current. The photometer electronics was the same as used previously (Björn 1971 b). The methods for calibration of the photometer are described elsewhere (Björn 1971 c). For light of wavelength 740 nm calibration by the "electric method" with 24 volt lamps gave a sensitivity of 159 unit · m^2 · μW^{-1}, while comparison with a bolometer gave the value 149 unit · m^2 · μW^{-1}. The average of both methods is 154 unit · m^2 · μW^{-1}. In the present experiments the sensitivity was increased 155 times by removing the diaphragm placed immediately in front of the photomultiplier so that the whole cathode area ($4.15 \cdot 10^{-4}$ m^2) was exposed. Thus the sensitivity with respect to radiation leaving the sample was $154 \cdot 155/12 \cdot 4.15 \cdot 10^{-4}$ unit · μW^{-1} = 4.80 unit · pW^{-1} (at 740 nm).

For a computation of the radiant energy and the number of photons from the sample, the emission spectrum has to be known. Experiments with filters indicated that the spectrum of the light leaving the sample has its maximum at a wavelength greater than 720 nm, probably at about 740 nm. The same is the case for the slow, far-red induced afterglow from a cuvette filled with *Elodea* leaves. The reason for the long wavelength of the maximum is that some of the light produced is reabsorbed within the sample, and that this reabsorbtion decreases with wavelength (cf. Virgin 1954). The "true" (undistorted) spectrum from a thin sample could not be measured because of the faintness of the alcohol-induced luminescence. It is, however, likely to be similar to the fluorescence spectrum. The latter was measured on a leaf from near the shoot apex with an Aminco-Bowman spectrophotofluorometer equipped with the R 374 photomultiplier (a special adapter was required) shielded by a 2 mm OG2 glass filter (Schott & Gen.). The excitation monochromator was set at 430 nm and the exciting light filtered through a 2 mm BG12 glass filter (Schott & Gen.). Readings were taken every 10 nm and corrected by use of the spectral response curve determined previously (Björn 1971 c). The resulting fluorescence spectrum (Figure 1) is in fair agreement with that published by Virgin (1956) for an *Elodea* leaf. It can be calculated from the spectrum that 1 J of fluorescence light corresponds to $3.53 \cdot 10^{18}$ photons.

The spectrum was used for the luminescence determinations in the following way: for a certain interference filter with a transmission maximum at 740 nm the quantity

$$\int_{630\text{ nm}}^{790\text{ nm}} T(\lambda)F(\lambda)d\lambda \Big/ \int_{630\text{ nm}}^{790\text{ nm}} F(\lambda)d\lambda$$

was determined. In this expression, $F(\lambda)$ stands for the ordinate in the fluorescence energy spectrum at wavelength λ and $T(\lambda)$ is the fraction of incident light of wavelength λ transmitted by the filter. The ratio of the above integrals was 0.0487. This is the fraction of energy with the spectral distribution of undistorted fluorescence that would penetrate the interference filter. In experiments with isobutanol-induced luminescence from a cuvette packed with 100 leaves, insertion of the interference filter between sample and photomultiplier decreased the photometer reading to 10.7 percent of that without interference filter. If it is assumed that luminescence within the wavelength band around 740 nm is not reabsorbed by the sample, the factor 0.107/0.0487 = 2.20 should be used to correct the measured luminescence for self-absorption in experiments without filter, and the photometer sensitivity for 740 nm should be used. In other words, the effective photometer sensitivity is 4.80/2.20 = 2.18 units per pW of radiation generated in the sample, or $6.18 \cdot 10^{-7}$ unit · s · $photon^{-1}$.

The above computation is rather crude. The spectra for fluorescence and alcohol-induced luminescence may differ. The sample is not completely transparent even at 740 nm. The transmission of the interference filter was measured in a spectrophotometer with the light passing the filter perpendicularly, and the photometer was

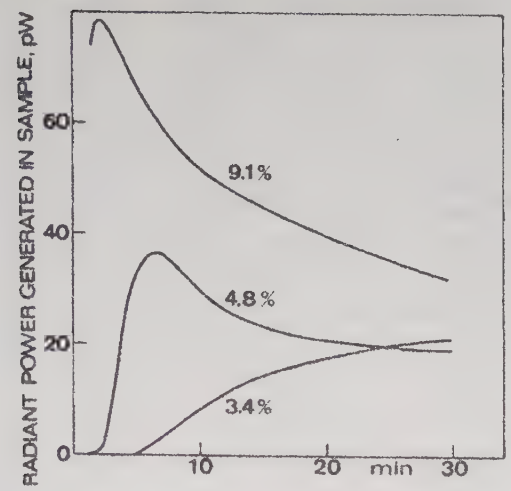


Figure 2. *Luminescence induced by isobutanol of different concentrations.* Ordinate: Radiant power generated per 0.4 µmol chlorophyll. Abscissa: Time after the addition of the substance. The samples were aerated.

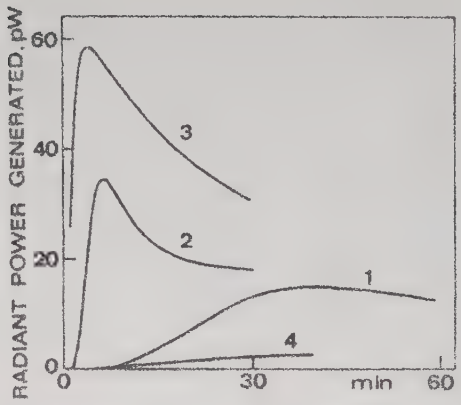


Figure 3. *Luminescence induced by various substances*: 25 % methanol (1), 4.8 % 1-butanol (2), 1 % 1-heptanol (3), and approx. 8.3 *M* formaldehyde (4). The figure shows individual experiments while each value in Table 1 is an average for two experiments. Otherwise as in Figure 2.

calibrated with light hitting the photocathode perpendicularly, while a wide angle is involved in the luminescence measurement.

A sample of 100 *Elodea* leaves contains about 0.4 micromole chlorophyll, but the chlorophyll content varies somewhat. To faciliate comparisons between different experiments, all luminescence values have been converted to luminescence per 0.4 micromole of chlorophyll. For instance, in the experiment shown in Figure 4 the sample had a chlorophyll content of 0.462 µmol, and the original readings have been multiplied by 0.4/0.462.

Leaves were prepared for electron microscopy as described elsewhere (Björn 1971 b) and the preparations were examined in a Philips EM 300 electron microscope.

Results

Luminescence was induced in *Elodea* leaves by every one of fifteen tested alcohols and three tested aldehydes, provided the concentration of the substance was high enough. Figure 2 shows the effect of various concentrations of isobutanol. With a low concentration there is a pronounced lag phase between the addition of the alcohol and the onset of light emission, and no maximum

Table 1. *Comparison of the effectiveness of various substances in inducing luminescence in Elodea leaves.*

Substance	No. of carbon atoms per molecule (n + 1)	Concentration		Thermodynamic activity (A)	Maximum radiant flux, pW	Time for reaching maximum, minutes	Radiant energy, nJ (0–30 min) (E)	E/A, nJ	E(0.2554)n/C		
		percent by volume	molarity of water phase (C)						alcohols		aldehydes
									prim.	sec.	
methanol	1	25	6.19	0.199	13.3	36.5	16.8	85	2.72	—	—
ethanol	2	25	4.27	0.240	43.2	9.5	82.8	345	4.93	—	—
2-propanol	3	25	3.27	—	13.3	7.0	36.0	—	—	0.72	—
1-propanol	3	9.1	1.18	0.316	20.2	8.3	52.6	167	2.91	—	—
2-butanol	4	9.1	0.992	—	17.0	10.3	45.4	—	—	0.76	—
1-butanol	4	4.8	0.525	0.528	37.2	5.0	82.6	156	2.62	—	—
isobutanol (2-methyl--1-propanol)	4	4.8	0.532	—	34.6	7.0	75.1	—	2.35	—	—
1-hexanol	6	1.0	$5.77 \cdot 10^{-2}$	1	49.3	3.0	101.5	102	1.91	—	—
1-heptanol	7	1.0	$1.46 \cdot 10^{-2}$	1	60.2	2.3	155.5	156	2.96	—	—
1-octanol	8	1.0	$3.76 \cdot 10^{-3}$	1	47.3	1.8	115.9	116	2.18	—	—
1-decanol	10	1.0	$2.34 \cdot 10^{-4}$	1	37.1	6.5	95.0	95	1.88	—	—
formaldehyde	1	—	~8.3	(steady incr.; 3.7 pW at 45 min)			5.6	—	—	—	0.68
acetaldehyde	2	9.1	1.62	—	17.0	24.0	42.3	—	—	—	6.67
propanal	3	4.8	0.666	—	28.7	5.8	71.8	—	—	—	7.03

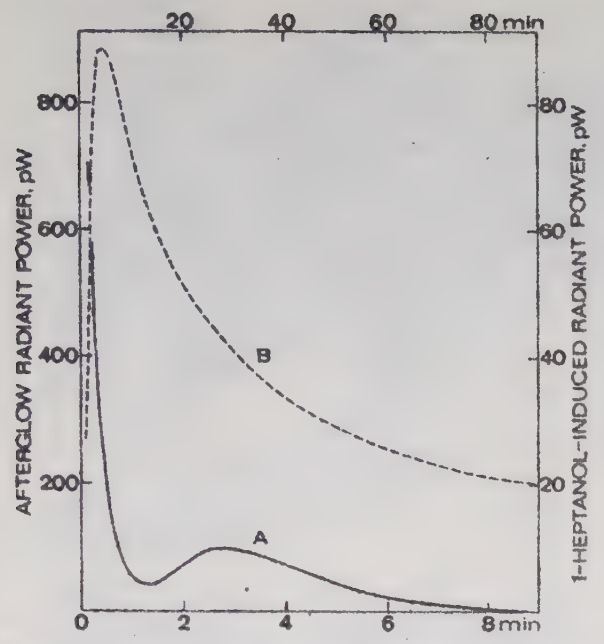


Figure 4. *Comparison for the same sample of the maximum afterglow inducеable by far-red light* (curve A, left ordinate and lower abscissa) *and luminescence induced by undiluted 1-heptanol* (curve B, right ordinate and upper abscissa). The areas under the curves are directly comparable. The heptanol induced luminescence continued for much longer than the diagram shows. The radiant flux was 16.2 pW 140 min and 5.95 pW 780 min after the addition of heptanol. The total luminescence energy was estimated by linear extrapolation through the two latter measurements and addition of the triangle obtained to the actually measured part.

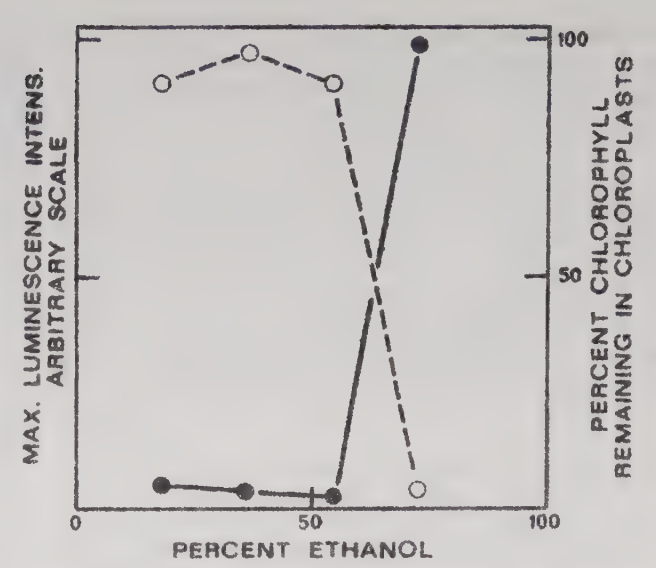


Figure 5. *Maximum intensity of luminescence from spinach chloroplasts treated with ethanol* (solid line, left ordinate) *and percent of the chlorophyll remaining in the chloroplasts after 15 minutes.* Abscissa: Ethanol concentration, percent by volume.

was recorded during the time of observation. With a high concentration, on the other hand, light emission starts within a few seconds, rapidly rises ta a maximum, and then declines. The same behavior was observed with other alcohols, but the concentrations required for the different types of response differed. It is difficult to make a quantitative comparison between the effectiveness of different alcohols. The same concentration could not be used throughout, since the lower alcohols produce no light at all at a low concentration, while the higher alcohols have a limited solubility in water. After some preliminary experiments is was decided to make tests with four different concentrations, 25, 9.1, 4.8 and 1.0 percent by volume. The higher alcohols are not soluble even to a concentration of 1 percent, and the experiments were carried out with emulsions (see Materials and Methods).

Luminescence curves for three alcohols and formaldehyde are shown in Figure 3, and results for other substances are summarized in Table 1 which shows average values for duplicate experiments.

In addition to these substances, 1-pentanol, isoamylalcohol (3-methyl-1-butanol), tert. amylalcohol (2-methyl-2-butanol) and 1-hexanol were found to induce considerable luminescence, but only single experiments were made. n-hexane and acetone were also tested; only very faint luminescence was obtained in these latter cases.

To determine more exactly the yield of radiation that could be obtained under favorable conditions, a sample of 100 *Elodea* leaves was immersed in undiluted 1-heptanol, and the luminescence was monitored for 13 hours. The luminescence during the first 90 minutes of the experiment is shown in Figure 4. For comparison, the diagram also shows the afterglow from the same sample measured before the heptanol experiment under conditions of saturating excitation with far-red light (Björn 1971 b). It is readily seen that the total amount of luminescence obtainable with alcohol treatment greatly exceeds that obtainable by far-red irradiation, even if the maximum intensity is far less.

The total luminescence up to infinite time was estimated by taking the area under the intensity curve from 10 seconds up to 140 minutes, and adding to this a triangle obtained by linear extrapolation through the values for 140 and 780 minutes down to the time-axis. The energy so obtained amounts to $8.24 \cdot 10^{-7}$ J per 0.4 µmol chlorophyll (actually $9.52 \cdot 10^{-7}$ J for a sample containing 0.462 µmol). Of this 8.0 percent is the emission estimated to take place after the end of measurements (later than 13 hours after the addition of heptanol). The estimate of this latter part is almost certainly

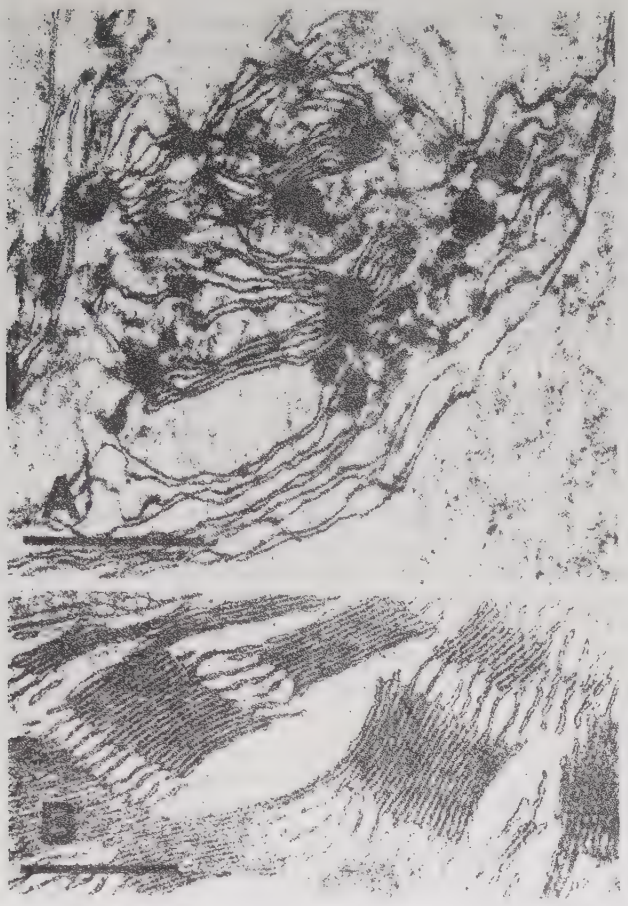


Figure 6. *Electron micrographs of an Elodea leaf treated for 7 minutes with 5 percent 1-butanol* (A) *and of a control leaf* (B). The bars indicate 500 nm. Permanganate fixation.

too low, but hardly by as much as an order of magnitude. By use of the fluorescence spectrum in Figure 1, $8.24 \cdot 10^{-7}$ J per 0.4 μmol chlorophyll is found to be equivalent to 1 photon in $8.28 \cdot 10^4$ chlorophyll molecules.

Only a very small fraction of the chlorophyll in the leaves had diffused to the external medium after 13 hours incubation with heptanol.

In an experiment to determine the effect of oxygen concentration on alcohol-induced luminescence, *Elodea* leaves were immersed in a two-phase mixture of water and 1-butanol, and the cuvette flushed alternately with air and nitrogen, while the oxygen tension in the medium was recorded. With air flushing, the values shown by the oxygen meter were too low (12 rather than 21 percent), obviously because of insufficient circulation of the liquid around the electrode. No effect on the luminescence was detected when nitrogen was flushed through the sample, while the oxygen reading fell to below one fifth of the original. In a sample treated with nitrogen prior to the addition of the alcohol, luminescence developed and decayed more rapidly and showed a higher maximum than for an aerated sample, but there was no qualitative difference between the curves.

Luminescence was observed also from isolated chloroplasts when they were treated with alcohol. In Figure 5 the maximum intensity after ethanol addition is plotted against ethanol concentration. After 15 minutes the experiments were terminated, the suspensions centrifuged and the amounts of chlorophyll dissolved in the medium and remaining in the chloroplasts determined. The percentage of chlorophyll remaining in the chloroplasts after the different alcohol treatments is shown in Figure 5.

The following differences between the results with leaves and with isolated chloroplasts are worth mentioning: much higher external ethanol concentration was required to induce luminescence in isolated chloroplasts, and light emission was coupled with the appearance of chlorophyll in the medium. The luminescence rose and decayed more rapidly with chloroplasts. Some aromatic compounds (thymol, α-naphthol), which had no effect on whole leaves, induced luminescence in chloroplasts at a low concentration (1/4 saturated water solution).

To determine the structural effect of alcohol treatment, an *Elodea* leaf was immersed for 7 minutes in 5 percent 1-butanol and then fixed with permanganate. An electron micrograph of the preparation together with a control (leaf fixed without previous alcohol treatment) is shown in Figure 6. In the butanol-treated leaf lipid drops have formed at the edges of the former grana. The whole lamellar system has become disordered but not completely disintegrated.

Discussion

There are many examples of chemiluminescence occurring when organic substances are oxidized. Since no effect on the luminescence intensity was observed when the oxygen concentration was varied, it is not likely that the luminescence studied here belongs to this class of phenomena. (The lack of oxygen effect on alcohol-induced luminescence is in contrast to the oxygen dependence of far-red induced afterglow, Björn 1971 a). The experiments indicate that the light emission is a physical phenomenon associated with the dissolution of chlorophyll. The fact that hexane induces only very weak luminescence can be explained by its insolubility in water and the impermeability of the cells to the substance. No explanation for the low effectiveness of acetone has been found.

We have found no mention in the literature of luminescence accompanying the dissolution of a substance. On the other hand, there are many examples of crystallo-

luminescence, *i.e.* luminescence associated with crystallization of various substances. One example that is worth mentioning in this connection is the luminescence that has been claimed (Lenard *et al.* 1928) to occur when aqueous solutions of sodium chloride or potassium chloride are mixed with ethanol. A test of this reaction (with sodium chloride) in our laboratory was, however, completely negative. It may be that certain impurities must be present to form luminescence centers. In any case, this kind of alcohol-induced luminescence has nothing to do with that from leaves and chloroplasts, since the latter is red and no doubt emanates from chlorophyll.

Luminescence from leaves can be induced by media of lower ethanol concentration than that required for luminescence from isolated chloroplasts. The reason may be that the leaf cells actively concentrate the alcohol to levels of internal thermodynamic activity higher than of the medium. The reader should bear this in mind during the following discussion which uses arguments strictly valid only for thylakoid membranes in direct contact with the external medium. A rigorous quantitative test of some of the ideas will have to await further experiments with isolated chloroplasts and chloroplast fragments.

The relative effectiveness of various alcohols, considered on a concentration basis, is comparable to what has been found for other alcohol effects on biological membranes and model systems. Among well-investigated physiological effects, narcosis, permeability changes, and, to a certain extent, olfaction seem to be comparable. Particularly striking when the compounds are compared on the basis of concentration or dosage is the very low effectiveness of methanol, and an increase in effectiveness with increasing length of the carbon chain (Cherry *et al.* 1970, Gudjónsdóttir and Burström 1962, Siegel and Halpern 1964, cf. also Ferguson 1939 and Schneider 1968). Another common feature if luminescence induction and other alcohol effects on membranes seems to be the higher effectiveness of normal primary alcohols in relation to secondary isomers (Gudjónsdóttir and Burström 1962, Siegel and Halpern 1964, Skou 1958). To achieve a certain stimulation of cell extension in illuminated wheat roots, isobutanol was required at a ten times higher concentration than 1-butanol (Gudjónsdóttir and Burström 1962), while the two compounds were almost equally effective in the present experiments.

The concentration of the active substance in the aqueous medium, whether expressed as percentage or molarity, is questionable as a basis for expressing effectiveness. Several other ways have been proposed: thermodynamic activity in relation to pure liquid (Ferguson 1939) or in relation to some other standard state (Schneider 1968), and computed partial molecular volume of the substance in the membrane (Laffort 1965, Mullins 1954). In the first case low molecular alcohols are often found to have at least as high effectiveness as long-chain alcohols (Bangham *et al.* 1965, Cherry *et al.* 1970).

To consider the present results in relation to thermodynamic activity, the activity coefficients for solutions of methanol, ethanol, 1-propanol and 1-butanol at 25°C (Butler *et al.* 1933) were used since no values for 20°C were found in the literature. The activity coefficients were multiplied by the molar fractions to obtain the activities. For 1-hexanol and higher alcohols the activity was near unity, since the water phases were saturated; for the other substances the activities are unknown. The light energy measured during the first thirty minutes (E in Table 1) was divided by the activity (A). As is evident from Figure 2 this is a crude way of comparing the energy values. It underestimates the relative effectiveness of a substance tested under conditions such that a small amount of radiation is produced (in this case the luminescence during the first 30 minutes seems to be a smaller fraction of the total obtainable than is otherwise the case). No attention can therefore be paid to the low value of E/A for methanol. The high value of E/A for ethanol is, however, remarkable. With ethanol more luminescence was obtained than with 1-propanol, although the latter was used at a higher activity.

The drops visible in Figure 7 probably consist of the membrane lipids, solubilized by dissolved butanol. Haydon and Taylor (1963) have made theoretical predictions of the relations between the concentrations of various substances in the water phase required to break up bimolecular arrays of phospholipid molecules into drops. The threshold concentrations for two consecutive members of a homologous series (such as two primary alcohols differing by one $-CH_2-$ group) were found to differ approximately by the factor $e^{-400°K/T}$, which for the absolute temperature $T = 293°K$ (20°C) is equal to 0.2554. Since the light emission may be associated with drop formation, the emission induced by various alcohols $C_{n+1}H_{2n+3}OH$ and aldehydes $C_nH_{2n+1}CHO$ should be considered in relation to $C/(0.2554)^n$, where C is the molarity in the water phase. The value of C in the suspensions of 1-hexanol and higher alcohols was obtained from the solubility at 25°C (Kinoshita *et al.* 1958) since no values for 20°C were found. As shown in the last columns of Table 1, $E(0.2554)^n/C$ is fairly constant within each group of compounds (primary alcohols, secondary alcohols, and aldehydes). Compared in this way ethanol is more effective and formaldehyde less effective than their analogs. Aldehydes (with the exception of formaldehyde) are more effective than primary alcohols, which in turn are more effective than secondary alcohols.

From these considerations of the relative amounts of radiation produced, let us turn to the absolute amounts.

The maximum observed energy emission (with undiluted 1-heptanol) was $8.24 \cdot 10^{-7}$ J for 0.4 μmol chlorophyll or $3.4 \cdot 10^{-24}$ J per chlorophyll molecule. With 1 nm² of membrane surface per chlorophyll molecule (Kreutz 1966) this means $3.4 \cdot 10^{-24}$ J per nm² of membrane surface.

When drops like those in Figure 7 A are formed, the lipid-aqueous interfacial area in the chloroplast lamellar system decreases. No values for the interfacial tensions in such interfaces are available, but it seemed worth while to make an estimate to be able to compare the decrease in interfacial free energy with the light emission.

Interfacial tensions for organic substances in contact with water range from zero (for substances completely miscible with water and for phospholipid, see below) to about 50 dyn · cm^{-1} for paraffins such as n-hexane and n-octane (Washburn 1928). The only value for plant lipids we have found is about 30 dyn · cm^{-1} for refined cottonseed oil (Kirk and Othmer 1965). Many measurements of surface pressures of monomolecular chlorophyll films spread on water have been carried out; the value for a tightly-packed film of chlorophyll a on water or on a dilute aqueous buffer is about 22 (Bellamy *et al.* 1963) to 30 (Trurnit and Colmano 1959) dyn · cm^{-1}. If we use 26 dyn · cm^{-1} for the film pressure and 73 dyn · cm^{-1} as the surface tension of water, we find the sum of the tensions in the chlorophyll-water and chlorophyll-air interfaces to be 73–26 = 47 dyn · cm^{-1}. This is in agreement with other esters (Washburn 1928) for which values of 42 to 60 dyn · cm^{-1} can be computed. For the esters for which are known, the ester-water interfacial tension values range from 16 to 24 dyn · cm^{-1}, so 20 dyn · cm^{-1} is a reasonable guess for chlorophyll. The thylakoid membranes contain other lipids in addition to chlorophyll, some of which have strongly polar groups. These groups are likely to be directed towards the aqueous phase and thus markedly decrease the interfacial tension. The latter is therefore likely to be below 20 dyn · cm^{-1} = $2 \cdot 10^{-22}$ J · nm^{-2} which should be regarded as an upper limit of the interfacial free energy. Allowing for two lipid-aqueous interfaces of each lipid layer, this would mean a maximum of $4 \cdot 10^{-22}$ J · nm^{-2}. If we count only one interface per lipid layer (the other one masked by proteins) and, as a low estimate of the interfacial tension, use the lowest listed (Washburn 1928) for any compound with at least 5 carbon atoms (excepting phospholipids, 2.73 dyn · cm^{-1} for isovaleric acid) we arrive at a minimum free energy of $2.7 \cdot 10^{-23}$ J · nm^{-2}. Since the interfacial area of the drops is negligible compared to that of the original membrane system, $2.7 \cdot 10^{-23}$ J · nm^{-2} is also a minimum estimate of the free energy decrease of the system. One should add to this, however, the decrease in free energy of the alcohol molecules when they enter the lipid. It is readily seen that the available energy exceeds the observed emission at least by the factor 8.

The discussion above has limited validity for membranes containing phospholipids, since these substances have rather special properties. They may have zero interfacial tension towards water; for pure phospholipid in water the most stable configuration which is spontaneously attained is a bimolecular array (Haydon and Taylor 1963). To get an estimate of the energy that can be released by alcohol addition to water in contact with a membrane containing phospholipid, one may consider the experiments of Skou (1958). He used a natural mixture of lipids and measured the increase in surface pressure of monomolecular films of constant area on water to which alcohols were added. Assuming the film to behave ideally, the pressure increase from 10 dyn · cm^{-1} to 19.3 dyn · cm^{-1} corresponds to 19.3 $\ln \frac{19.3}{10}$ dyn · cm^{-1} = $1.27 \cdot 10^{-22}$ J · nm^{-2} to be obtained as mechanical energy if the film were allowed to expand. This is 37 times as much as the maximum light energy measured in our experiments, and it is obtainable with alcohol concentrations much lower than in our experiments (*e.g.* 0.240 *M* 1-propanol, 0.438 *M* 2-propanol or 0.080 *M* 1-butanol).

The comparisons above show only that interfacial energy is large enough to account for the light emission, not that this energy transformation actually takes place. The luminescence may be quanta from light energy trapped in special deep traps or "blind alleys". These could be molecules of special long-wavelength forms of chlorophyll a turned into short-wavelength fluorescent forms by being dissolved in the organic phase.

If the luminescence is created by transformation of interfacial energy, the energy to be emitted as a photon from a chlorophyll molecule must be collected from an appreciable membrane area. The observed emission corresponds to only a few photons per thylakoid. The average luminescence photon carries $2.84 \cdot 10^{-19}$ J, which corresponds to $2.2 \cdot 10^{3}$ nm² in Skou's experiments.

Arnold (1966) has shown that *Chlorella* cells which are heated without preillumination show thermoluminescence peaks at 20°C, 55°C and 95°C. It is possible that the peak at 55°C is associated with the melting and rearrangement of membrane lipids. If this is the case, this kind of thermoluminescence is closely related to alcohol-induced luminescence.

Several persons at the Department of Zoology at the University of Lund have contributed to the present investigation. In particular I wish to thank Mrs. Lena Svenre for embedding and sectioning, and Dr. Tiit Kauri for help at the microscope.

References

Arnold, W. 1966. Light reaction in green plant photosynthesis. A method of study. — Science 154: 1046–1049.

Bangham, A. D., Standish, M. M. & Miller, N. 1965. Cation permeability of phospholipid model membranes: Effect of narcotics. — Nature 208: 1295–1297.

Bellamy, W. D., Gaines, G. L., Jr. & Tweet, A. G. 1963. Preparation and properties of monomolecular films of chlorophyll a and pheophytin a. — J. Chem. Phys. 39: 2528–2538.

Björn, L. O. 1971 a. Far-red induced, long-lived afterglow from photosynthetic cells. Size of afterglow unit and paths of energy accumulation and dissipation. — Photochem. Photobiol. 13: 5–20.

— 1971 b. Effects of some chemical compounds on the slow, far-red induced afterglow from leaves of Elodea, and lack of effect of N-methyl phenazonium methosulfate on far-red induced glucose uptake in Chlorella. — Physiol. Plant. 25: 316–323.

— 1971 c. Simple methods for the calibration of light measuring equipment. — Physiol. Plant. 25: 300–307.

Butler, J. A. V., Thomson, D. W. & Maclennan, W. H. 1933. The free energy of the normal aliphatic alcohols in aqueous solution. 1933. — J. Chem. Soc. 1933: 674–686.

Cherry, R. J., Dodd, G. H. & Chapman, D. 1970. Small molecule — lipid membrane interactions and the puncturing theory of olfaction. — Biochem. Biophys. Acta 211: 409–416.

Ferguson, J. 1939. The use of chemical potentials as indices of toxicity. — Proc. Roy. Soc. London 127 B: 387–404.

Gudjónsdóttir, S. & Burström, H. 1962. Growth-promoting effects of alcohols on excised wheat roots. — Physiol. Plant. 15: 498–504.

Haydon, D. A. & Taylor, J. 1963. The stability and properties of bimolecular lipid leaflets in aqueous solutions. — J. Theoret. Biol. 4: 281–296.

Kinoshita, K., Ishikawa, H. & Shinoda, K. 1958. Solubility of alcohols in water determined by the surface tension measurements. — Bull. Chem. Soc. Japan 31: 1081–1084.

Kirk, R. E. & Othmer, D. F. (eds.). 1965. Encyclopedia of Chemical Technology. — 2nd ed. 8: 788. Interscience Publishers. New York, London, Sydney.

Kreutz, W. 1966. On the state of chlorophyll in vivo. — Zschr. Naturforsch. 23 b: 520–527.

Laffort, P. 1965. Efficacité odorante et activité thermodynamique. — Rev. Laryng. 1965: 860–879.

Lenard, P., Schmidt, F. & Tomaschek, R. 1928. Phosphoreszenz und Fluoreszenz. — 2. Teil, p. 981. *In* Handb. d. Experimentalphysik (ed. W. Wien & F. Harms), vol. 23. — Akad. Verlagsgesellschaft, Leipzig.

Mayne, B. C. 1968. The light requirement of acid-base transition induced luminescence of chloroplasts. — Photochem. Photobiol. 8: 107–113.

— & Clayton, R. K. 1966. Luminescence of chlorophyll in spinach chloroplasts induced by acid-base transition. — Proc. Natl. Acad. Sci. 55: 494–497.

Mullins, L. J. 1954. Some physical mechanisms in narcosis. — Chem. Rev. 54: 289–323.

Schneider, H. 1968. The intramembrane location of alcohol anesthetics. — Biochem. Biophys. Acta 163: 451–458.

Seeman, P., Sha'afi, R. I., Galey, W. R. & Solomon, A. K. 1970. The effect of anesthetics (chloropromazine, ethanol) on erythrocyte permeability to water. — *Ibid.* 211: 365–368.

Siegel, S. M. & Halpern, L. A. 1964. The effect of branching at C-1 on the biological activity of alcohols. — Proc. Natl. Acad. Sci. 51: 765–768.

Skou, J. C. 1958. Relation between the ability of various compounds to block nervous conduction and their penetration into a monomolecular layer of nerve-tissue lipoids. — Biochem. Biophys. Acta 30: 625–629.

Strehler, B. L. & Arnold, W. 1951. Light production by green plants. — J. Gen. Physiol. 34: 809–820.

Trurnit, H. J. & Colmano, G. 1959. Chloroplast studies I. Absorption spectra of chlorophyll monolayers at liquid interfaces. — Biochem. Biophys. Acta 31: 434–447.

Virgin, H. I. 1954. The distortion of fluorescence in leaves by light scattering and its reduction by infiltration. — Physiol. Plant. 7: 560–570.

— 1956. Some notes on the fluorescence spectra of plants in vivo. — *Ibid.* 9: 674–681.

Washburn, E. W. (ed.) 1928. International Critical Tables. — 1st ed. 4: 436–437. McGraw-Hill New York.

Factors Affecting Ethanol-Induced Luminescence in Dark-Treated Chloroplasts

By

BJÖRN SIGFRIDSSON

Department of Plant Physiology, Fack, S-220 07 Lund, Sweden

(Received 2 March, 1978; revised 14 June, 1978)

Abstracts

Dark-treated chloroplasts emit light when treated with a high ethanol concentration. The ethanol treatment causes chlorophyll solvation. The light is red and emanates from a singlet excited molecule, probably a chlorophyll peroxide. It is quenched by acetone, sodium dodecyl sulfate treatment of the chloroplasts before the addition of ethanol, boiling, reducing substances and low pH. It is enhanced by ferricyanide and high pH. This is interpreted as a requirement for an organized structure and for an energy transfer system for light emission to occur.

Introduction

Photosynthetic material will emit light after illumination (after-glow or delayed light emission, Strehler and Arnold 1951). This is due to de-excitation of excited chlorophyll, created by an inversed energy-depending electron flow. Thus, treatments impeding the electron transport chain or the energy-holding capacity will influence the luminescence. To date, the requirement for energy has been met by light treatment or by the addition of ATP (Schreiber and Avron 1977) under proper conditions. The luminescence can be triggered by adding salts, pH changes, rapid temperature increase, electric fields (see Hardt and Malkin 1972 for references) or by organic solvents (Hardt and Malkin 1972).

Besides their triggering effect, alcohols will induce light emission from intact *Elodea* not preilluminated or treated with ATP. Ethanol will induce luminescence from not preilluminated or ATP treated spinach chloroplasts (Björn and Sigfridsson 1971). However, it must be added in such a high concentration that it certainly had disorganizing effects. It was considered interesting to learn, if this type of luminescence depends on remnants of electron transport and on the energy-holding capacity of the intact chloroplast.

Abbreviations: ATP, adenosine triphosphate; A_λ, absorbance at wavelength λ in a 1 cm cuvette; B, an electron carrier between PQ and Q; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PQ, plastoquinone; PS II, photosystem II; Q, the primary electron acceptor of PS II; SDS, sodium dodecyl sulfate; Tris, tris-(hydroxymethyl)-aminomethane.

Materials and Methods

Chloroplast preparation: 60 g deribbed leaves from spinach (*Spinacea oleracea* L. cv. Viking, Weibulls, Landskrona, Sweden) grown in soil in a greenhouse were homogenized in a mortar with sand and 60 ml of a solution containing 0.35 *M* NaCl, 12 m*M* ascorbic acid and 20 m*M* tris-HCl pH 8.2. After filtration through four layers of gauze the homogenate was centrifuged for 2 min at 200 *g*, and the supernatant centrifuged again for 7 min at 1000 *g*. The sedimented chloroplasts were washed once in the above solution and resuspended in about 5 ml of a resuspension medium containing 0.35 *M* NaCl and 20 m*M* tris-HCl pH 8.0. The suspension was stirred for 1 h. The entire procedure was performed at 5°C and the suspension stored at 5°C for 2 to 4 days until used. Luminescence was almost independent of the chloroplast age within this time interval.

Determination of chlorophyll: An aliquot of the chloroplast suspension was extracted with 80% acetone. After centrifugation (3 min at 1000 *g*) the absorbance A_λ of the supernatant was determined at 645, 663 and 700 nm. The Chl concentration was computed according to the following formula based on the absorbance coefficients of MacKinney (1941):

$$\text{Chl} = [0.905(A_{663} - A_{700}) + 2.224(A_{645} - A_{700})] \cdot 10^{-5}\ M$$

Subtraction of apparent absorbance at 700 nm decreases error due to slight turbidity.

Luminescence measurements: The luminometer setup was essentially as previously described (Björn and Sigfridsson 1971). To increase the signal-to-noise ratio most measurements were made with a rotating disk chopper between sample and photomultiplier, and the latter (a Hamamatsu R 374) was connected to a lock-in amplifier. The signal was smoothed by an integrating circuit with a time constant of

100 s and then fed to a chart recorder. In a typical experiment a chloroplast suspension containing 0.6 mg Chl was diluted to 2 ml with resuspension medium. The sample was kept for 20 min in complete darkness in the sample compartment of the luminometer before addition of 9.0 ml 99.5% ethanol. To avoid illumination of the chloroplasts this addition was made with a hypodermic syringe through a small hole in the lid of the sample compartment. All luminescence measurements were made at about 20°C.

Other chemical additives to the chloroplast suspension were, if possible, administered as solutions in resuspension medium adjusted to pH 8.0. If they could not be dissolved in this way, they were administered as small volumes of concentrated ethanol solutions. No external light was allowed to reach the chloroplasts during addition of these substances, which was made either 5 min before or 15 min after the addition of ethanol. Blank experiments were made with the addition of solvent without dissolved substance. The concentrations of additives reported are the final concentrations, *i.e.* concentrations after the addition of both test substance and luminescence-inducing ethanol to the chloroplast suspension.

Digitonin treatment: Chloroplasts in resuspension medium were incubated with digitonin (0.5% by weight) in darkness at 5°C for 30 min prior to ethanol addition.

Triton X-100 treatment: Chloroplasts initially suspended in 0.35 *M* NaCl and 50 m*M* tris-HCl pH 7.8 were resuspended in 0.5 *M* sucrose and 50 m*M* tris-HCl, pH 7.5, to a concentration of 2–2.5 mg Chl per ml. Triton X-100 was added to a concentration of 1 g per 40 mg Chl. The solution was stirred for 1 h at about 5°C. The suspension was stored for 2–4 days before use.

SDS treatment: Chloroplasts in resuspension medium were incubated with SDS in darkness for 5 min prior to ethanol addition. The molar ratio of SDS to Chl was 125 to 1.

Absorption measurements: Absorption measurements were made with an Aminco DW-2 UV/VIS spectrophotometer.

Fluorescence measurements: Fluorescence measurements were made with an Aminco-Bowman Spectrophotofluorometer with slit combination 4 (half band with about 15 nm) and an R-136 photomultiplier. Sample concentrations were kept so low that fluorescence values were proportional to Chl concentrations. Chl fluorescence was excited with blue light (430 nm) passed through a 2 mm glass filter (BG 12, Schott & Gen.) before reaching the sample. The photomultiplier was protected from stray blue light by a 2 mm glass filter (RG 1, Schott & Gen.).

pH buffering: For measurements above pH 8.0 the tris-HCl buffer (20 m*M*) was used for the resuspension medium. pH values below 8 were obtained with a 20 m*M* phosphate buffer (with traces of tris-HCl buffer from the resuspended chloroplasts).

Heat treatment: Chloroplasts were boiled for 5 min before being put into the luminometer.

Chemicals: All chemicals were of analytical grade. They were used in the form delivered by the manufacturer.

Results

The amount and decay of luminescence varied with the amount of ethanol added (Figure 1). When resuspension medium, or 29% ethanol were added, only a small amount of

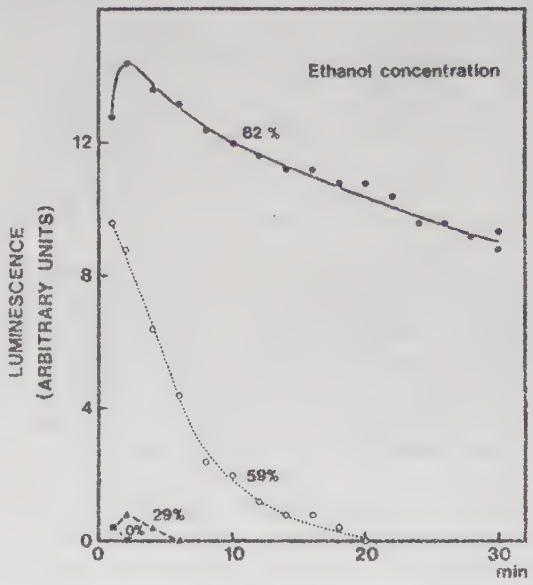


Figure 1. *Luminescence from chloroplasts treated for 20 min in darkness.* Resuspension medium, pH 8.0. Addition of ethanol to 0, 29, 59 and 82% at time zero. 0, 29 and 59% ethanol were obtained by diluting 99.5% ethanol with resuspension medium, pH 8.0.

luminescence was obtained. Addition of 59% ethanol resulted in light emission which decayed within 20 min. With 82% ethanol there was considerable light emission even after 30 min. Absorption spectra measurements on the different solutions showed that 59 and 82% ethanol extracted the chlorophyll from the membranes.

Because of the weakness of the luminescence, only a rough estimate of the spectral composition could be made by means of the 2 mm glass cut-off filters (Schott & Gen.) available. The light intensity was unaffected by an RG 1 filter (50% transmission at 605 nm), decreased to about 60% by a 670 nm filter and to about 40% by a 695 nm filter. The spectral sensitivity of the particular photomultiplier used was not determined. Calibration of another Hamamatsu R 374 photomultiplier showed that the sensitivity decreased by a factor of 2 between 650 and 750 nm (Björn and Sigfridsson 1971).

The luminescence was quenched by copper chloride and copper acetate but not by cobalt chloride or potassium acetate after the ethanol addition (Figure 2). However, the chlorophyll fluorescence was not influenced by any of the salts. Reducing substances such as dithionite or ascorbate

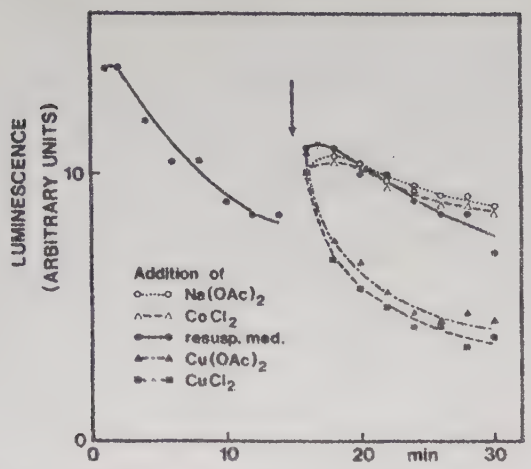


Figure 2. *Effect of copper chloride* (10^{-4} *M*), *copper acetate* (10^{-4} *M*), *cobalt chloride* (10^{-4} *M*) *and potassium acetate* (2×10^{-4} *M*) *on luminescence from chloroplasts treated for 20 min in darkness.* Resuspension medium, pH 8.0. The salts were added in 1 ml resuspension medium (pH adjusted to 8.0 when necessary), 15 min (arrow) after the addition of ethanol to 82% at time zero.

enhanced the intensity of the luminescence during the first minutes, but it soon decayed and the total light emission was diminished when they were added before the ethanol (Figure 3). When they were added after the ethanol, the light intensity was decreased (not shown in Figure 3). The oxidizing substance ferricyanide increased the intensity and the total amount of light given off when added before (Figure 3) or after the ethanol (not shown in Figure 3). Chl fluorescence was not influenced by these substances.

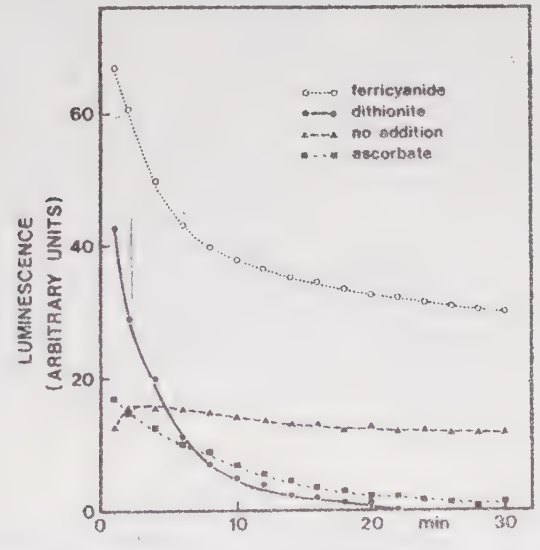


Figure 3. *Influence of ferricyanide* (1 m*M*), *ascorbate* (1 m*M*) *and dithionite* (1 m*M*) *on luminescence from chloroplasts treated for 20 min in darkness.* Resuspension medium, pH 8.0. The substances were added in 1 ml resuspension medium (pH adjusted to 8.0 when necessary) 5 min before the addition of ethanol to 82% at time zero.

Table 1. *Influence of some treatments on the ethanol-induced luminescence*

Treatment	Before or after the ethanol addition	Results
DCMU (5×10^{-6} *M*) + NH_2OH (3×10^{-3} *M*)	Before or after	No effect
NH_2OH (10^{-2} *M* or 5×10^{-5} *M*)	Before or after	No effect
NaN_3 (12×10^{-3} *M*)	After	No effect
m-Dinitrobenzene (20×10^{-3} *M*)	Before or after	Quenching
β-Carotene (1.5×10^{-3} *M*)	Before or after	No effect
Acetone (12%)	After	Quenching
Digitonin (0.5%)	Before	No effect
Triton X-100 (1 g per 40 mg Chl)	Before	No effect
SDS (125 mol per mol Chl)	Before	Quenching
Boiling (5 min)	Before	Quenching

The luminescence was not inhibited by DCMU (5×10^{-6} *M*) and NH_2OH (3×10^{-3} *M*) added simultaneously, before or after the addition of ethanol (Table 1). NH_2OH at high (10^{-2} *M*) or low (5×10^{-5} *M*) concentration did not inhibit luminescence when added before or after the addition of ethanol. Potassium azide, 12 m*M*, a quencher of singlet oxygen (Deneke and Krinsky 1977) added after the ethanol addition did not influence the luminescence. The luminescence was quenched by *m*-dinitrobenzene, a quencher of singlet states (Seely 1966, p. 534, citing Evstigneev *et al.*) but not by *β*-carotene, a quencher of triplet states (Fujimori

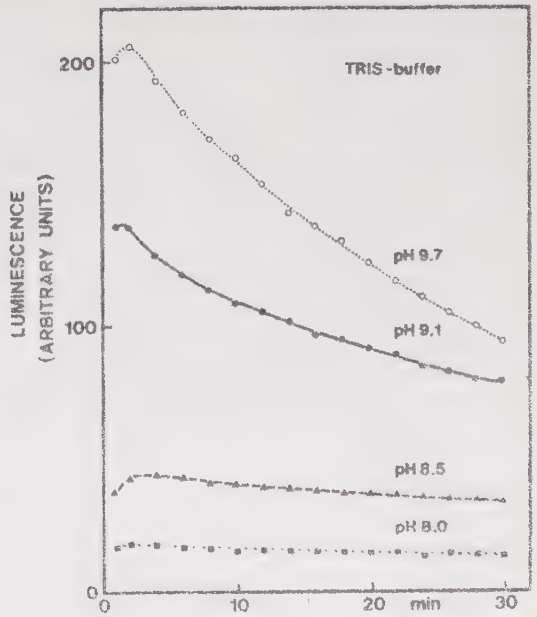


Figure 4. *Effect of pH 8.0, 8.5, 9.1 and 9.7 on luminescence from chloroplasts treated for 20 min in darkness.* The pH values were obtained by adjusting the tris-HCl buffer in the resuspension medium. Ethanol were added at time zero to 82%.

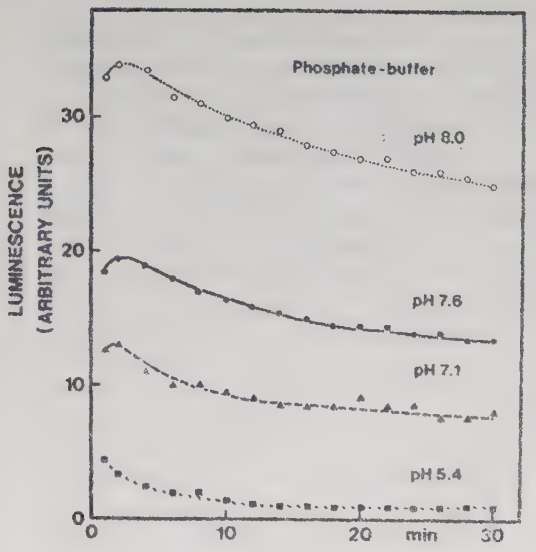


Figure 5. *The result of pH 8.0, 7.6, 7.1 and 5.4 on luminescence from chloroplasts treated for 20 min in darkness.* The pH values were obtained with a 20 m*M* phosphate buffer in the resuspension medium. Ethanol were added at time zero to 82%.

and Livingston 1957). Acetone inhibited light emission even at low concentrations when added after the ethanol. This is not due to a dilution of the ethanol which is present in a concentration well above the one needed to induce luminescence. The luminescence was not affected by the detergents digitonin or Triton X-100. (However, a particular batch of Triton X-100, about 3 years old, induced luminescence when used without the addition of ethanol. The reason for this was not further investigated. It could be due to some impurity in the particular batch or to some decomposition product.) SDS treated chloroplasts emitted less light than untreated ones when ethanol was added. Boiled chloroplasts emitted only a small amount of light. The light intensity 1 min after ethanol addition was about 30% of that from unboiled ones. The light emission declined soon and was insignificant after about 15 min.

The pH of the chloroplast suspension prior to ethanol addition influenced light emission markedly. High pH values enhanced light emission compared to low ones (Figures 4 and 5). Addition of alkali after the ethanol enhanced light emission, while addition of acid decreased it. However, the chlorophyll fluorescence was not influenced by changes in pH.

Discussion

The origin of luminescence from higher plants is a recombination of an electron hole pair in PS II. The formation of the electron hole pair is due to an energy-depending reversed electron transport. Addition of various substances to preilluminated chloroplasts can trigger the light emission. So is the case for ethanol (Hardt and Malkin 1972). In this case the luminescence is quenched by DCMU in combination with NH_2OH, since DCMU blocks the electron transport from PQ to Q and NH_2OH reduces the holes on the oxidizing side of PS II.

A way to produce a charge separation is to add DCMU to dark-treated chloroplasts. This causes a lowered oxidation-reduction potential of B (Bouges-Boquet 1973) between Q and PQ. Now B can reduce Q and thus luminescence will be possible (Etienne and Lavorel 1975). However, in chloroplasts stored in darkness for more than 40 min, DCMU cannot induce luminescence. The luminescence of this type is quenched by NH_2OH in low or high concentrations.

The ethanol-induced luminescence cannot be of any of the types described above. In the first system a preillumination is required and the luminescence is quenched by DCMU in combination with NH_2OH. Neither of these characteristics are valid for the ethanol-induced luminescence. In the second system there is also a requirement for a preillumination within the last 40 min, before the addition of DCMU. So is not the case for the ethanol-induced luminescence, which can be induced from chloroplasts kept in the dark for 60 min. The ethanol-induced luminescence is not quenched by low or high concentrations of NH_2OH, and not stimulated by DCMU. Taken together, this must mean that ethanol extracts components in the electron transport chain which will mediate the effect of NH_2OH and DCMU on the charge separation preceding the luminescence. Also, the energy for the luminescence does not come from a pool previously loaded with energy by preillumination.

Krasnovskii Jr. and Litvin (1974) studied luminescence from chlorophyll in organic solvents, among others ethanol and acetone. According to them, the light emanates from decomposing chlorophyll peroxides, and the luminescence requires preillumination. The reaction mechanism seems to be the following: Singlet excited chlorophyll, arising when the chlorophyll solution is illuminated, is converted to triplet chlorophyll. This reacts with oxygen giving singlet oxygen. Singlet oxygen in turn reacts with chlorophyll giving a chlorophyll peroxide, which on decomposition gives rise to the light emitter. This system shows both similarities and differences to the system studied here. In both, chlorophyll is in solution in an organic solvent, and the light is emitted by a singlet excited molecule. The luminescence is in both systems quenched by copper ions, known to deactivate chlorophyll peroxides (Krasnovskii and Litvin 1969). Although no emission spectra could be taken for the ethanol-induced luminescence, the result with the cut-off filters showed that the emitted light is red. It could thus be supposed that the luminescence in the ethanol system is due to the decomposition of a chlorophyll peroxide giving rise to singlet excited chlorophyll. However, the chlorophyll peroxide could not arise in the same way in the two systems, since in the ethanol system there is no need for a preillumination, although there is in the system of Krasnovskii Jr. and Litvin.

Besides that, NaN_3, a quencher of singlet oxygen, has no influence on the ethanol-induced luminescence. However, the energy needed to excite chlorophyll must be provided.

According to the chemiosmotic hypothesis (Mitchell 1961, 1966), chloroplasts store energy in form of a proton gradient across their membranes. Although pH influenced luminescence induced by ethanol, it is not probable that the energy comes from a proton gradient across the membranes, since the high concentration of ethanol needed to induce luminescence certainly has destroyed the structural basis for this type of energy storage. Since boiling and the strong detergent SDS, but not the milder ones such as digitonin and Triton X-100, quenches luminescence, it is tempting to suggest some lipoprotein involved in the luminescence. This lipoprotein structure could deliver or mediate the energy needed to excite chlorophyll. The pH effect could be due to its influence on the configuration of proteins. The quenching effect of acetone could be due to its different action on protein precipitation compared to ethanol (Huang *et al.* 1971). The effect of oxidizing and reducing substances could be due to their influence on the energy transfer to the chlorophyll.

The author is much indebted to Professor L. O. Björn for advice and criticism during the investigation. A. H. Bjarnasson and J. Th. Jónsson constructed one of the luminometers as a course project under the supervision of Dr. L. Grahm, Department of Electrical Measurements, University of Lund.

References

Björn, L. O. & Sigfridsson, B. 1971. Luminescence induced in intact leaves and isolated chloroplasts by alcohols and other substances. — Physiol. Plant. 25: 308–315.

Bouges-Bocquet, B. 1973. Electron transfer between the two photosystems in spinach chloroplasts. — Biochim. Biophys. Acta 314: 250–256.

Deneke, C. F. & Krinsky, N. I. 1977. Inhibition and enhancement of singlet oxygen ($^1\Delta_g$) dimol chemiluminescence. — Photochem. Photobiol. 25: 299–304.

Etienne, A. L. & Lavorel, J. 1975. Triggered-luminescence in dark adapted Chlorella cells and chloroplasts. — FEBS Lett. 57 (3): 276–279.

Fujimori, E. & Livingston, R. 1957. Interactions of chlorophyll in its triplet state with oxygen, carotene, etc. — Nature 180: 1036–1038.

Hardt, H. & Malkin, S. 1972. Luminescence of isolated chloroplasts induced by organic solvents. — Biochim. Biophys. Acta 276: 588–594.

Huang, K. H. & Tao, M. C. & Boulet, M. & Riel, R. R. & Julien, J. P. & Brisson, G. J. 1971. A process for the preparation of leaf protein concentrates based on the treatment of leaf juices with polar solvents. — J. Inst. Can. Technol. Aliment. 4 (3): 85–90.

Krasnovskii, A. A. & Litvin, F. F. 1969. Thermoluminescence of solutions of chlorophyll and its analogues after illumination at low temperature. — Mol. Biol. (Mosc.) 3 (2): 282–293.

Krasnovskii, A. A., Jr. & Litvin, F. F. 1974. Long-lived afterglow of photosynthetic pigments in solutions: Photochemiluminescence. — Photochem. Photobiol. 20: 133–149.

MacKinney, G. 1941. Absorption of light by chlorophyll solutions. — J. Biol. Chem. 140: 315–322.

Mitchell, P. 1961. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. — Nature 191: 144–148.

— 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. — Biol. Rev. 41: 445–502.

Schreiber, U. & Avron, M. 1977. ATP-induced chlorophyll luminescence in isolated spinach chloroplasts. — FEBS Lett. 82 (1): 159–162.

Seely, G. R. 1966. Photochemistry of chlorophylls in vitro. — *In* The Chlorophylls (L. P. Vernon and G. P. Seely, ed.), pp. 523–568.

Strehler, B. L. & Arnold, W. 1951. Light production by green plants. — J. Gen. Physiol. 34: 809–820.

Some Effects of Humidity on the Light Reaction of Photosynthesis in the Lichens *Cladonia impexa* and *Collema flaccidium*.

by

Björn Sigfridsson
University of Lund
Department of Plant Physiology
Box 7007
S-220 07 Lund 7
Sweden

Accepted for publication in Physiologia Plantarum

Abstract

Luminescence and fluorescence were measured at different relative humidities in the lichen Cladonia impexa Harm. Both parameters showed a constant value at relative humidities lower than 80%; thereafter they increased. Low relative humidity was found to decrease energy transfer from chlorophyll b to chlorophyll a, inhibit electron transport, lower electron holding capacity on the reduced side of photosystem two and impair far-red-induced afterglow, reflecting photophosphorylation. In Collema flaccidium Ach. Ach. energy transfer from the accessory pigments phycocyanin and phycoerythrin to chlorophyll a was only possible at high relative humidity. The results are interpreted as due to conformational changes caused by changed water content in the lichen.

Introduction

Lichens are often exposed to water stress. When the lichen is dry, metabolism is reduced to a low level. However, under favourable conditions lichens rapidly regain metabolism. For such a system to function, it seems probable that there is some kind of switch, turning the water sensitive processes on or off.

Since photosynthesis is a vital process for the lichen and since it is known to proceed only when RH in the surrounding air is high (Bertsch 1966), photosynthesis could be expected to be regulated by the proposed switch. To investigate this, a technique measuring primary behaviour in the photosynthetic apparatus is necessary. Hitherto, photosynthesis in lichens has been measured by manometric techniques, oxygen electrode or infrared gas analyzer. All these techniques suffer from disadvantages. The lichens have often been exposed to unfavourable conditions during measurements, and it is difficult to account for respiratory activity in both the alga and the fungus (Richardson 1973). Other pitfalls such as photorespiration have been quietly forgotten (Farrar 1973). Techniques circumventing these drawbacks are luminescence and fluorescence measurements, but the relationship of these to photosynthesis must be determined. The aim of this investigation was to study such relationships and determine the usefulness of luminescence and fluorescence in investigating the effect of water potential on the light reaction of photosynthesis.

Abbreviations: ATP, adenosine triphosphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; IR, infrared; $NADPH_2$, reduced nicotinamide adenine dinucleotide phosphate; PM, photomultiplier; PQ, plastoquinone; PS I, photosystem one; PS II, photosystem two; Q, the primary electron acceptor of PS II; RH, relative humidity.

Materials and Methods

Plant material

Two species of lichen were used, Cladonia impexa Harm. and Collema flaccidium Ach. Ach. The former contains a green alga (Trebouxia sp.) and the latter a blue-green (Nostoc sp.). The lichens were collected in Skåne, Sweden, and used the year around. Cladonia was used within a month after collection. It was stored at room temperature, daylight and about 50 % RH. Negative effects of storage could not be detected. Collema was used immediately after collection.

RH regulation

During an experiment the lichens were placed in air-tight plexiglass cuvettes of about 500 ml volume. RH was adjusted to the desired value by 80 ml sulfuric acid of proper concentration (Stokes and Robinson 1949) at the bottom of the cuvette, below the lichen. The lichen was allowed to equilibrate for 24 h at 25°C. During this time the plexiglass cuvette was exposed to an irradiance of about 9 $W \cdot m^{-2}$ for 18 h.

DCMU treatment

Lichens, treated at 100 % RH, were soaked for 30 min in 10^{-5} M DCMU solution. The DCMU was dissolved in ethanol before being added to the water. The final ethanol concentration was 1.25 % by volume. After DCMU treatment the lichens were equilibrated to 50 % RH for 24 h at about 20°C, before being exposed to the desired RH value. To account for the influence of soaking, control experiments were done in which lichens were soaked in water with an ethanol concentration of 1.25 % by volume, but without DCMU.

Luminescence and steady state fluorescence measurements

For measuring luminescence and fluorescence the apparatus in Figure 1 was used. Light from a Sylvania 500 W halogen lamp was filtered through a 5 cm thick water layer, a 5 cm thick layer of 0.3 M $CuSO_4$ and appropriate filter/s (Schott & Gen.). For details see the individual experiments. The light was focused on the lichen thallus with the aid of lenses and mirrors. By means of a rotating disk which either allowed illumination of the sample or opened for PM 1 (Hamamatsu R 374 photomultiplier protected by a 2 mm RG 5 glass filter), luminescence was measured 3 ms after excitation. When the rotating disk was stopped in a position allowing constant illumination of the lichen, fluorescence could be measured by PM 2 (Hamamatsu R 374 photomultiplier), since part of the fluorescence was reflected to PM 2 by a transparent glass plate. A 688 nm interference filter (half-band width 16 nm) and a 2 mm RG 665 glass filter were inserted in front of PM 2. Since stray light could not be completely avoided, a blank experiment with no sample in the cuvette was subtracted from the measured luminescence and fluorescence signals.

Fluorescence kinetics and far-red light-induced luminescence measurements

The plexiglass cuvette was placed in a light-tight outer metal cuvette (Figure 2). Excitation light was filtered through appropriate filters, when necessary complemented by interference filters (for details see the individual experiments). The photomultiplier was a Hamamatsu R 374 protected by glass filters, when necessary complemented by an interference filter (for details see the individual experiments). Mechanical shutters were used to regulate the exposure time for excitation and the measuring time for emission.

Fluorescence spectra

Fluorescence spectra were obtained with an Aminco-Bowman Spectrofluorometer equipped with slit combination 4 and a R-136 photomultiplier. The excitation light was filtered through a 2 mm BG 12 glass filter or a 2 mm OG 550 glass filter, and the photomultiplier was protected by a 2 mm RG 1 glass filter. The spectra were corrected for wavelength-dependent sensitivity of the equipment. All measurements were performed at about 20°C.

For all luminescence and fluorescence measurements, the series started and ended with 100 % RH. Only experiments in which there was good agreement between these two measurements (indicating that the lichens were not harmed by exposure to the sulfuric acid) are considered. The measurements were performed at 25°C, if not otherwise stated.

Results

Luminescence of Cladonia impexa, measured 3 ms after excitation, changed quickly when RH of the surrounding air was changed from 100 to 90 % (Figure 3). The intensity of the luminescence signal was halved within 5 h. When a dry thallus at 50 % RH was equilibrated to 100 % RH (Figure 3), the response time was slightly longer, with a short induction period of 2 to 3 h. After about 10 h the two curves fluctuated around a more or less constant value. However, luminescence values measured 24 h apart were fairly constant, which points to the presence of a circadian rhythm. From this experiment it was concluded that 24 h could be used as an equilibration time. To avoid errors due to possible circadian rhythms, all measurements were made at the same time of day.

Luminescence was constant between 50 and 80 % RH, whereafter it

rose with increasing RH (Figure 4). The increase was particularly pronounced between 90 and 95 % RH and did not approach zero at low RH. For the experiment in Figure 4 a young piece of lichen was used. If older samples were used the pattern was the same, but the amplitude less. This is certainly due to the lower parts of the lichen gradually dying away. Therefore in the following only young samples and as uniform as possible were selected for experiments. The degree of reproducibility is visualized in Figure 5 and Table 1 where standard errors are given.

The results in Figure 4 raised two questions. First, is luminescence composed of more than one component, and if so, what is the relation of each to photosynthesis? Secondly, since at least part of the luminescence is related to the photosynthetic process, how is this changed by RH?

ATP and $NADPH_2$ are not formed if photosynthesis is inhibited. This may happen (a) if transfer of light energy from the pigments absorbing the incident radiation to the reaction centra is hindered, (b) if the electron transport chain is blocked or (c) if the high energy state necessary for ATP production is prevented either from being developed or from being used. To test the first possibility, the following two experiments were done. Cladonia thalli, containing the green alga Trebouxia sp., were equilibrated with different values of RH. Fluorescence emission was measured at 688 nm with excitation at 425 nm or 483 nm. These wavelengths are preferentially absorbed by chlorophyll a or chlorophyll b, respectively. The ratios of fluorescence yield with excitation at 425 nm and 483 nm were calculated. This ratio decreased when RH increased, as shown in Figure 5 where the ratio was put to 100 at 70 % RH. This means that energy transfer from chlorophyll b to the fluorescent chlorophyll a decreased with decreasing RH.

A decrease in energy transfer from accessory pigments to chlorophyll *a* was also shown with decreasing RH for *Collema*, which contains a blue-green alga (Figure 6). When chlorophyll *a* was selectively excited (430 nm), there was only one peak at about 690 nm in the fluorescence spectrum, both at high and low RH. Excitation of phycocyanin (580 nm), however, resulted in peaks at about 690 nm and 660 nm at high RH, whereas only the 660 nm peak was seen at 50 % RH. Since chlorophyll *a* has a fluorescence emission peak at about 690 nm *in vivo*, the observations mean that energy reaches chlorophyll *a* by direct photon absorption both at low and high RH, but via phycocyanin only at high RH. At low RH energy transfer between phycocyanin and chlorophyll *a* is greatly diminished, and the absorbed energy is instead emitted as fluorescence at 660 nm from allophycocyanin.

Table 1 shows the effect of DCMU on luminescence and fluorescence from *Cladonia*. Both the luminescence and fluorescence signals were lower at 70 than at 100 % RH. When the thalli were pretreated with DCMU, luminescence decreased and fluorescence increased slightly at 100 % RH. At 70 % RH DCMU affected neither luminescence nor fluorescence. DCMU inhibits electron transport between Q and PQ, but has no effect at low RH. This means that low RH also inhibits electron transport, either on the oxidizing or the reducing side of PS II.

Fluorescence kinetics was used to measure electron storing capacity in the electron transport chain between PS II and PS I at different RH. The size of the area bounded by the fluorescence curve, the steady state fluorescence level and the ordinate can be attributed to the size of the electron acceptor pool on the reducing side of PS II (Zankel and Kok 1972). Thus it can be seen from Figure 7, that when RH

decreases, the electron storing capacity in the electron transport chain between PS II and PS I decreases. Notice also the lowering of fluorescence yield at decreasing RH.

To determine the influence of different values of RH on the energy coupling system, far-red-induced luminescence was measured. Luminescence decay showed a shoulder at 100 % RH 30-60 s after the actinic light was switched off (Figure 8), whereas lower RH did not give a shoulder. At low RH values luminescence intensity decreased drastically. This could be due to uncoupling of accessory pigments from the reaction centra of PS II.

The relationship between luminescence and fluorescence was measured at different RH. A linear relationship was found (Figure 9).

Discussion

Photosynthesis in lichens proceeds only at RH values higher than 80-85 %. This has been shown by IR measurements (Bertsch 1966). Luminescence from Cladonia shows a constant level at low humidities and increases above 80 % RH (Figure 4). Luminescence thus shows two components, one constant and not related to photosynthesis, and one variable, related to photosynthesis. The onset and inhibition of photosynthesis are rapid when humidity changes. When humidity is raised from 50 to 100 %, photosynthesis starts after 2 to 3 h and reaches half of its maximal level by 5 h (Figure 3). When humidity is lowered from 100 to 90 %, photosynthesis adapts equally fast (Figure 3). This points to an on-off mechanism, rather than to a destruction and rebuilding of components in the photosynthetic apparatus.

When humidity is lowered, there is a decrease in energy transfer from the light-harvesting pigments to PS II (Figures 5,6), in the capacity of electron transport (Table 1) and in electron holding capacity on the reduced side of PS II (Figure 7). Lowered humidity also influences the secondary maximum in far-red-induced luminescence, here reflected as a shoulder (Figure 8). The reason, why only a shoulder but not a maximum is obtained is probably due to the heterogeniously population of algae in the lichen, where some of the algae are in good physiological condition while others are not. Since only the former will give rise to a maximum and the latter not, the sum will come out as a shoulder. All these changes can be explained by conformational changes caused by the lowered water potential. In this context it could be relevant to mention that 80 % RH means a water potential of -306 bar at 25°C. Bertsch and Azzi (1965) claim that the maximum in far-red-induced luminescence is a reflexion of enzymatic back reactions coupled to the chlorophyll system. In "enzymatic back reactions" they obviously include the photophosphorylation system, which presumably in turn is dependent on conformational changes. Notice, that the shoulder has disappeared already at 95 % RH.

The lowered energy transfer between light-harvesting pigments and the fluorescent chlorophyll a of PS II could also be explained by conformational changes. In a model of the phycobilisome (Gray and Gantt 1974), allophycocyanin forms the connection with the thylakoid membrane which contains chlorophyll a. The extent of contact between the thylakoid membrane and the phycobilisome can vary (Harnischfeger and Codd 1978), which in the blue-green alga in Collema (Figure 6) can explain the changed energy transfer between phycocyanin and chlorophyll a, since energy transfer between pigments depends on their distance

and mutual orientation (Förster 1948). Changes in efficiency of energy transfer in blue-green algae have been shown for Anacystis nidulans, where energy transfer was influenced by the chlorophyll to phycocyanin ratio (Ghosh and Govindjee 1966). Changes in distance and orientation between chlorophyll b and chlorophyll a in the green alga in Cladonia could similarly result in changed energy transfer, as indicated in Figure 5. Whether the reduced electron transport and electron holding capacity are due to a block somewhere in the electron transport chain, or to decreased energy transfer to PS II at low RH cannot be decided as yet. If this is due to a block, it may not necessarily be identical to the DCMU block. A block on the oxidative side of PS II, inhibiting electron transfer to PS II, would give a similar result. In algae, electron transport between PS II and PS I is affected by desiccation. The sensitive site is most likely between PQ and P-700 (Wiltens et al. 1978).

When RH is decreased, both luminescence and fluorescence decrease (Figure 9). At low RH DCMU influenced neither luminescence nor fluorescence, but DCMU at high RH decreased luminescence and increased fluorescence (Table 1). This means that photosynthesis is inhibited in different ways by low RH and by DCMU at high RH. Since fluorescence at room temperature emanates from PS II and thus reflects its energy content, the lowered fluorescence at low RH can be due to (a) decreased initial input of quanta, (b) transfer of energy to PS I (spill-over) before fluorescence occurs (Butler 1978) or (c) increased heat deactivation. Sigfridsson and Öquist (1980) showed that energy distribution between the two photosystems is regulated by RH. As RH is decreased, excitation energy captured is preferentially distributed to PS I, instead of being reemitted as fluorescence from PS II, and thus fluorescence will de-

crease. Such changes in energy distribution may be caused by conformational changes due to altered water content of the membranes, which may lead to changes in distance and orientation between the pigments. Changes in orientation of only a few chlorophyll molecules can result in large changes in energy transfer between the two photosystems (Seely 1973).

Both luminescence and fluorescence are due to deactivation of excited chlorophyll molecules. However, the origin of their excitons differ. Luminescence excitons are created by a back reaction of the primary photo-oxidized and photoreduced products of the light reaction (Strehler and Arnold 1951) and are thus created via the PSII centra. The fluorescence excitons are created by light absorption anywhere in the pigment bed. Therefore, the question has been raised if they are "probes" that see the same chlorophyll territory (Lavorel 1975). As energy transfer between the photosynthetic pigments in lichens is RH sensitive, the territories the exitons can visit will also be RH dependent. Consequently, luminescence and fluorescence in lichens will reflect different processes at different RH, and their relation to photosynthesis must also be expected to be changed.

Acknowledgements

The author is much indebted to Professor L.O. Björn for advice and criticism during the investigation and to Dr. Gunnar Öquist for constructive criticism of the manuscript.

Figure legends

Figure 1. Apparatus used for measuring luminescence and fluorescence. For details see text and the individual experiments.

Figure 2. Apparatus used for measuring far-red-induced luminescence and induction of fluorescence. For details see text and the individual experiments.

Figure 3. Change in luminescence for Cladonia impexa with time when RH was changed from 100 % to 90 % (O- - - - O) and when RH was changed from 50 % to 100 % (●———●). For this experiment the apparatus in Figure 1 was used. The intensity of excitation light, filtered through a 2 mm BG 12 glass filter, was about 8 $W \cdot m^{-2}$.

Figure 4. Luminescence from Cladonia impexa at different RH values. For this experiment the apparatus in Figure 1 was used. The intensity of excitation light, filtered through a 2 mm BG 12 glass filter, was about 8 $W \cdot m^{-2}$.

Figure 5. Influence of RH on the ratio of fluorescence yield excited with 425 nm light (0.4 $W \cdot m^{-2}$) to that excited with 483 nm light (0.8 $W \cdot m^{-2}$) in Cladonia impexa. In this experiment the apparatus in Figure 1 was used. For 425 nm excitation a 2 mm BG 12 glass filter and a 425 nm interference filter (half-band width 17 nm) were inserted after the $CuSO_4$ filter. For 483 nm excitation a 2 mm BG 18 glass filter and a 483 nm interference filter (half-band width 15 nm) were used. Each point is the mean of six experiments. Vertical bars indicate standard errors (except for the value at 70 % RH, which was put to 100 for each individual experiment).

Figure 6. Fluorescence emission spectra for Collema flaccidium excited with (a) blue (430 nm; about 10 $W \cdot m^{-2}$) and (b) orange (580 nm; about 3 $W \cdot m^{-2}$) light, at high and low RH.

Figure 7. Induction of fluorescence in Cladonia impexa at different RH. For this experiment the apparatus in Figure 2 was used. The light source was a 20 W/6V Attralux spotlight. The excitation light was filtered through a 2 mm BG 12 glass filter, a 2 mm BG 18 glass filter and a 425 nm interference filter (half-band width 17 nm). The light intensity on the sample was about 0.1 $W \cdot m^{-2}$. The photomultiplier was protected by a 2 mm RG 665 glass filter and a 688 nm interference filter (half-band width 16 nm). The sample was left in darkness for 20 min before each measurements. The response time of the equipment was about 1 second.

Figure 8. Far-red-induced luminescence in Cladonia impexa at different RH. For this experiment the apparatus in Figure 2 was used. The light source was a 500 W Sylvania lamp. The excitation light was filtered through a 4 cm water bath, a heat absorbing glass filter, a 3 mm RG 715 glass filter and a 2 mm NG 9 glass filter. The radiation incident on the sample was about 70 $W \cdot m^{-2}$. The photomultiplier was protected by a 2 mm RG 665 glass filter. The excitation time was 1 minute. For each RH value the measurements were repeated until reproducible curves were obtained after 5-10 runs.

Figure 9. Relationship between fluorescence and luminescence of Cladonia impexa at different RH. For this experiment the apparatus in Figure 1 was used. The intensity of excitation light, filtered through a 2 mm BG 12 glass filter, was about 8 $W \cdot m^{-2}$. For each point the percentage of relative humidity is given.

Table 1. Influence of 10^{-5} M DCMU on fluorescence and luminescence from Cladonia impexa at 70 and 100 % RH. The intensity of fluorescence and luminescence was put to 100 at 100 % RH. Each value is the mean of four experiments. Standard errors are indicated.

RH	Fluorescence		Luminescence	
%	Sample 1	Sample 2	Sample 1	Sample 2
100	100	100	100	100
70	13.2 ± 6.72	17.2 ± 6.23	32.2 ± 3.42	33.5 ± 5.73
100	92.5 ± 9.60	97.0 ± 5.61	101.8 ± 8.72	99.6 ± 10.79
70	15.6 ± 6.22 + DCMU	18.5 ± 7.57 - DCMU	35.2 ± 1.69 + DCMU	35.2 ± 8.16 - DCMU
100	113.0 ± 9.07 + DCMU	97.2 ± 9.73 - DCMU	69.6 ± 4.05 + DCMU	95.8 ± 10.06 - DCMU

References

Bertsch, A. 1966. Ueber den CO_2-Gaswechsel einiger Flechten nach Wasserdampfaufnahme. - Planta 68:157-166.

Bertsch, W.F. & Azzi, J.R. 1965. A relative maximum in the decay of long-term delayed light emission from the photosynthetic apparatus. - Biochim. Biophys. Acta 94:15-26.

Butler, W.L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. - Annu. Rev. Plant Physiol. 29:345-378.

Farrar, J.F. 1973. Lichen physiology: progress and pitfalls. - In Air Pollution and Lichens (Ferry, B.W., Baddely, M.S. & Hawksworth, D.L., eds.) pp 238-282. Athlone Press, London. ISBN 0-485-11140-3.

Förster, Th. 1948. Zwischenmolekulare Energiewanderung und Fluoreszenz. - Ann. Phys. 2:55-75.

Ghosh, A.K. & Govindjee 1966. Transfer of the excitation energy in Anacystis nidulans grown to obtain different pigment ratios. - Biophys. J. 6:611-619.

Gray, B.H. & Gantt, E. 1974. Spectral properties of phycobilisomes and phycobiliproteins from the blue-green alga-Nostoc sp. - Photochem. Photobiol. 21:121-128.

Harnischfeger, G. & Codd, G.A. 1978. Factors affecting energy transfer from phycobilisomes to thylakoids in Anacystis nidulans. - Biochim. Biophys. Acta 502:507-513.

Lavorel, J. 1975. Luminescence. - In Bioenergetics of Photosynthesis (Govindjee, ed.) pp 223-317. Academic Press, New York, San Francisco, London. ISBN 0-12-294350-3.

Lavorel, J. & Joliot, P. 1972. A connected model of the photosynthetic unit. - Biophys. J. 12:815-831.

Myers, J. & Graham, J.-R. 1963. Enhancement in Chlorella. - Plant Physiol. 38:105-116.

Richardson, D.H.S. 1973. Photosynthesis and carbohydrate movement. - In The Lichens (Ahmadjian, V. & Hale, M.E., eds.) pp 249-288. Academic Press, New York and London. ISBN 0-12-044950-1.

Seely, G.R. 1973. Energy transfer in a model of the photosynthetic unit of green plants. - J. Theor. Biol. 40:189-199.

Sigfridsson, B. & Öquist, G. 1980. Preferential distribution of excitation energy into photosystem I of desiccated samples of the lichen Cladonia impexa and the isolated lichen-alga Trebouxia pyriformis. - Physiol. Plant.

Stokes, R.H. & Robinson, R.A. 1949. Standard solutions for humidity control at 25°C. - Industr. Engng. Chem. 41, p. 2013.

Strehler, B.L. & Arnold, W. 1951. Light production by green plants. - J. Gen. Physiol. 34:315-322.

Wiltens, J., Schreiber, U. & Vidaver, W. 1978. Chlorophyll fluorescence induction: an indicator of photosynthetic activity in marine algae undergoing desiccation. - Can. J. Bot. 56:2787-2794.

Zankel, K.L. & Kok, B. 1972. Estimation of pool sizes and kinetic constants. - In Methods in Enzymology (Colowick, S.P. & Kaplan, N.O., eds.) 24:218-238. Academic Press, New York and London.

Fig. 1

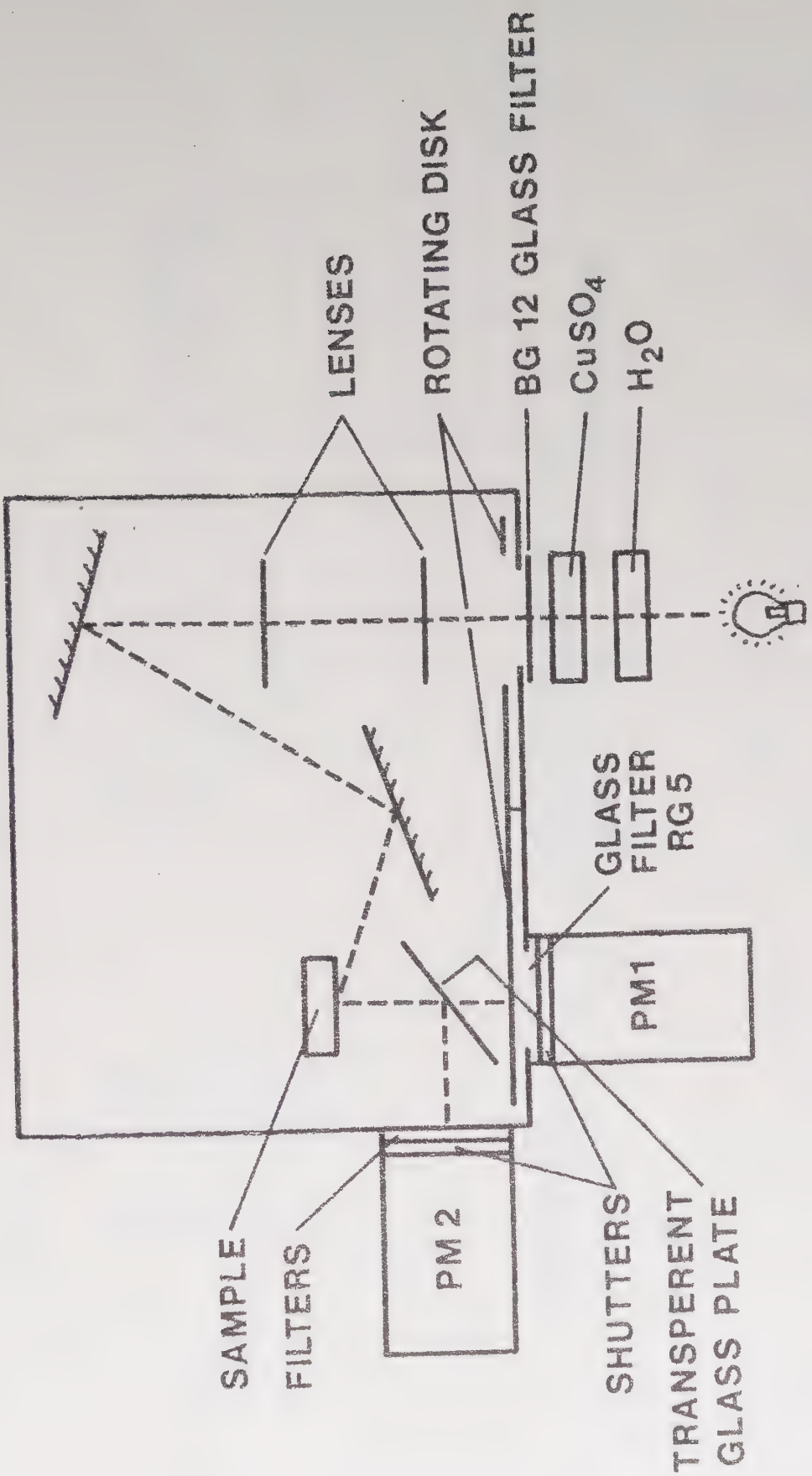

LENSES
ROTATING DISK
BG 12 GLASS FILTER
$CuSO_4$
H_2O
GLASS FILTER RG5
PM1
PM2
SAMPLE
FILTERS
SHUTTERS
TRANSPERENT GLASS PLATE

Fig. 2

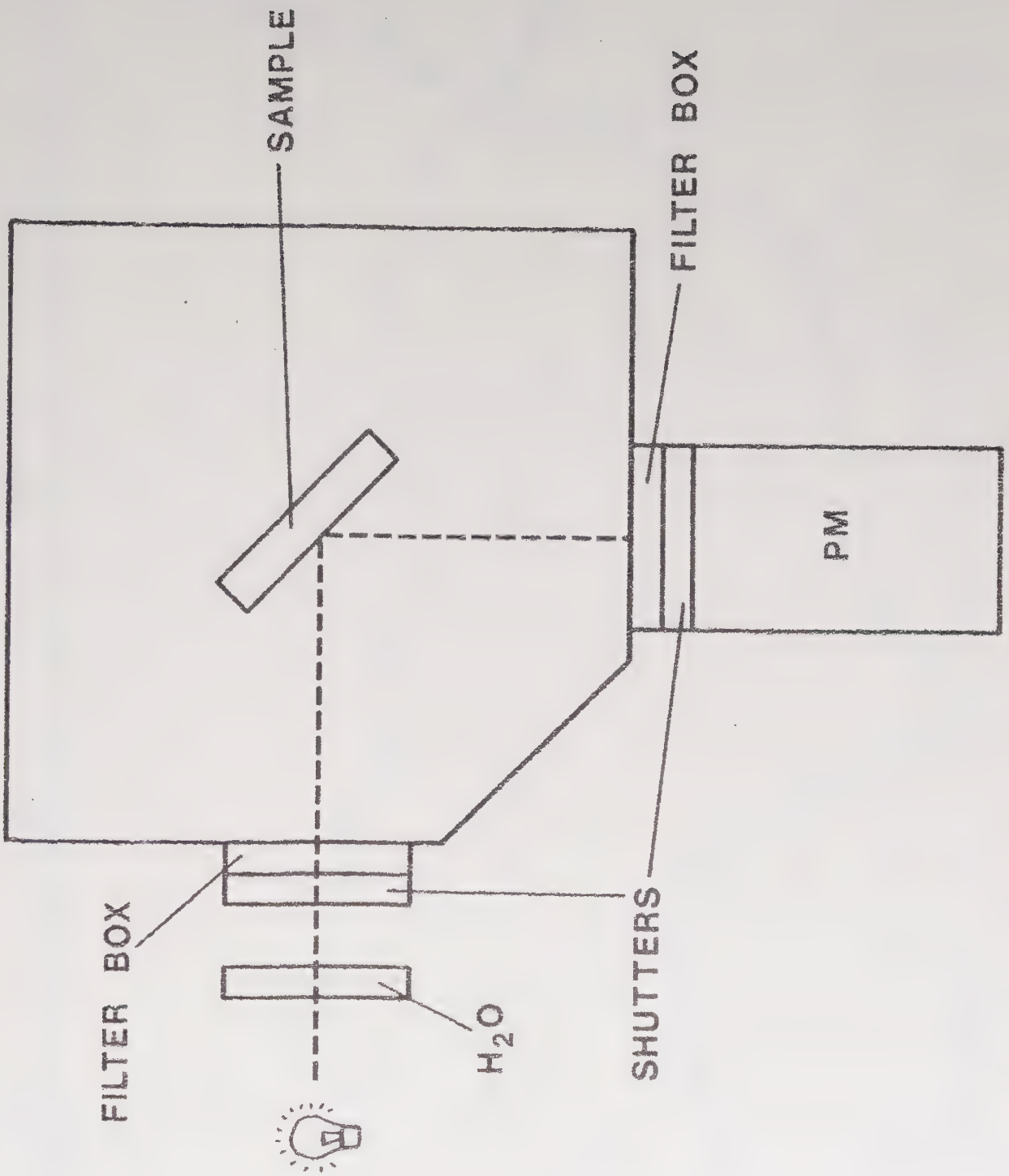
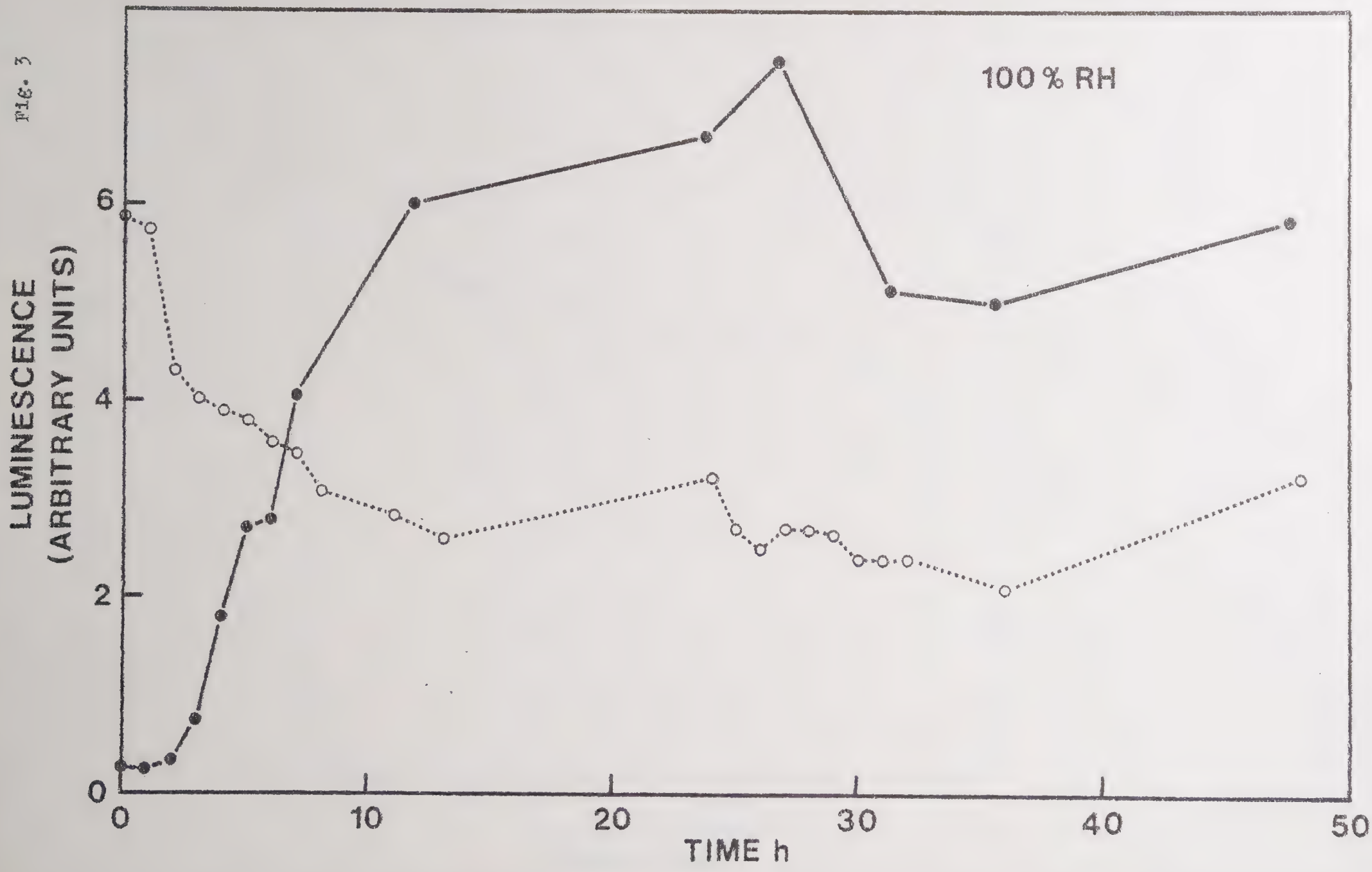
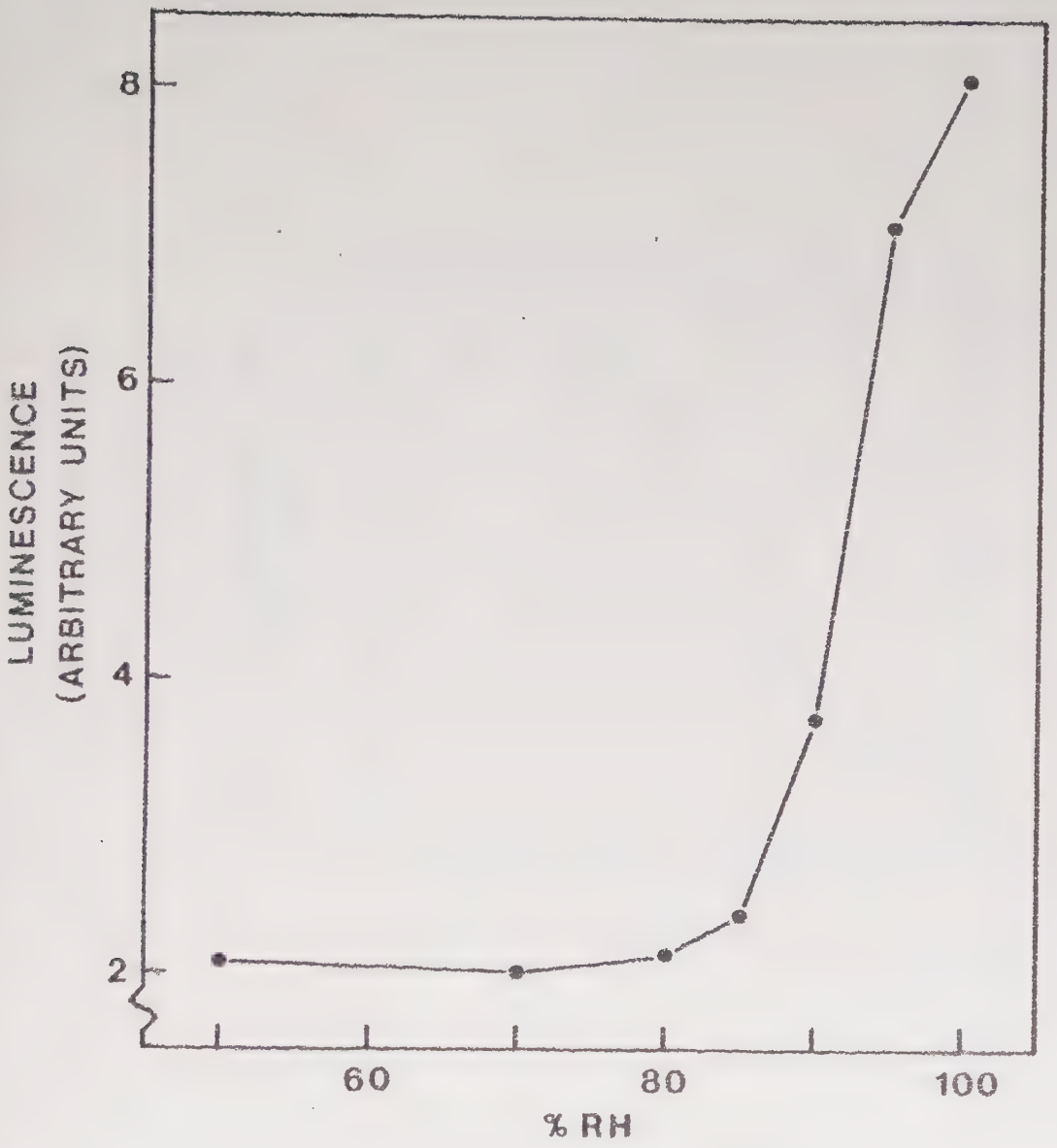
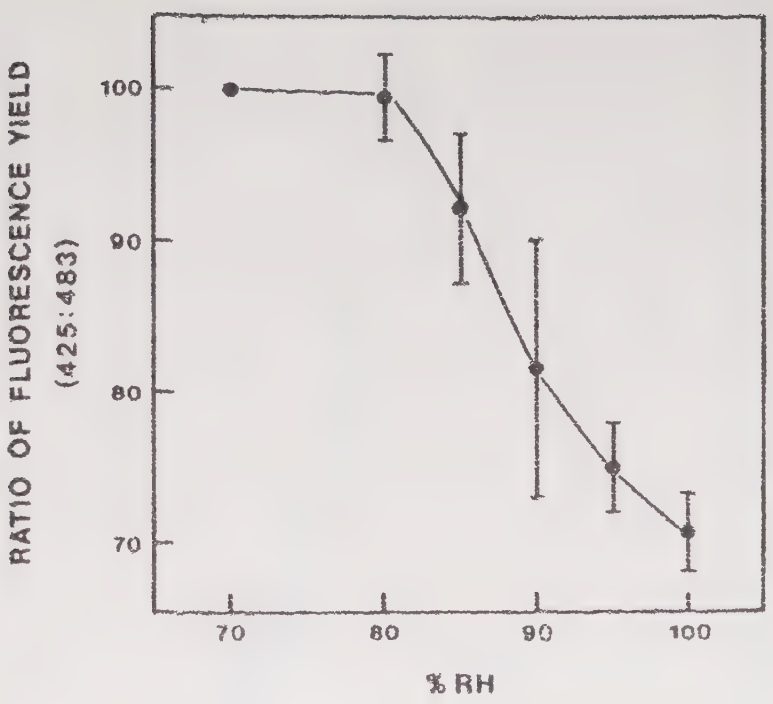
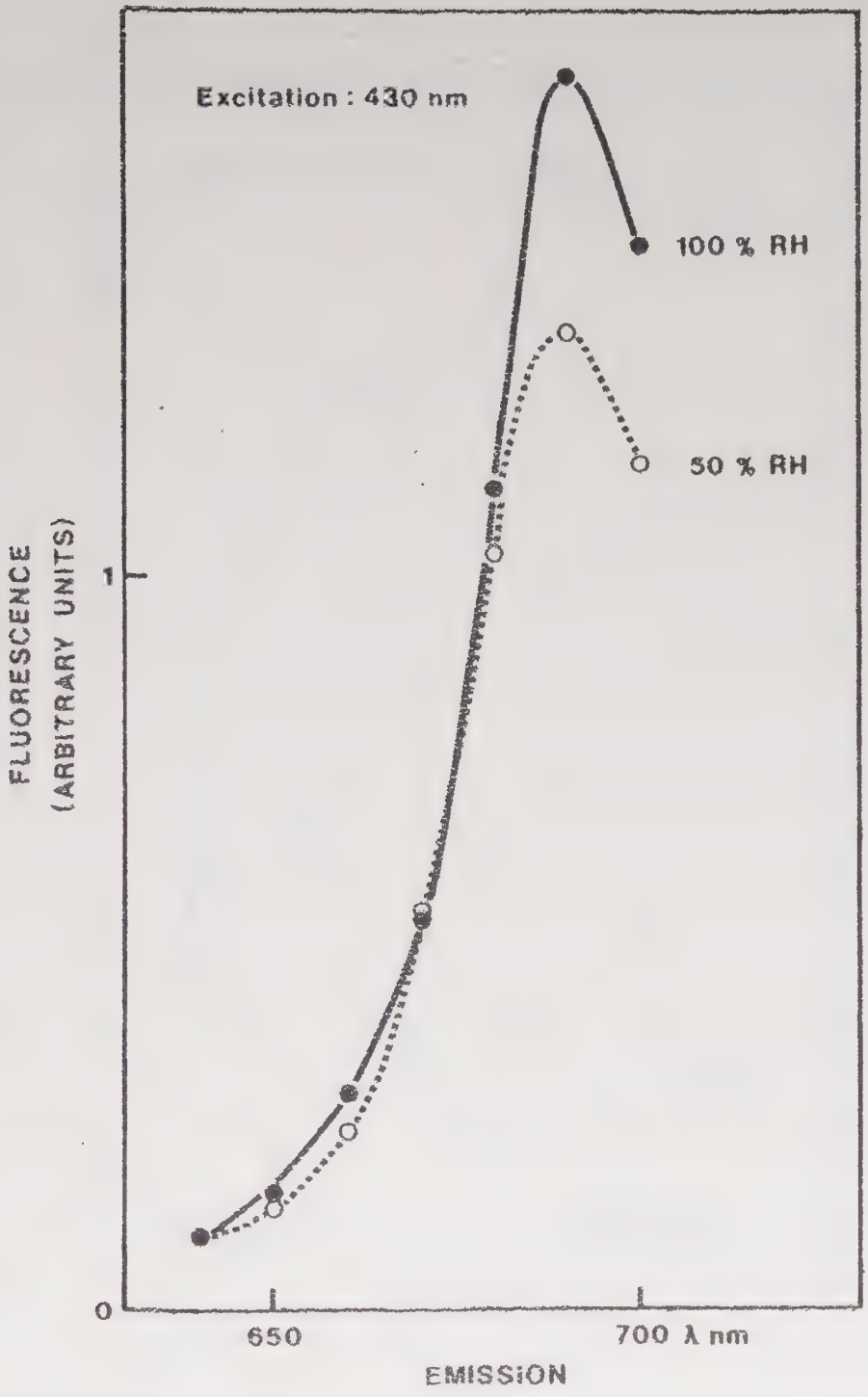


Fig. 3


100 % RH
LUMINESCENCE
(ARBITRARY UNITS)
0
2
4
6
0
10
20
30
40
50
TIME h


LUMINESCENCE
(ARBITRARY UNITS)
8
6
4
2
60
80
100
% RH


RATIO OF FLUORESCENCE YIELD
(425:483)
100
90
80
70
70
80
90
100
% RH


Excitation : 430 nm
100 % RH
50 % RH
FLUORESCENCE
(ARBITRARY UNITS)
1
0
650
700 λ nm
EMISSION

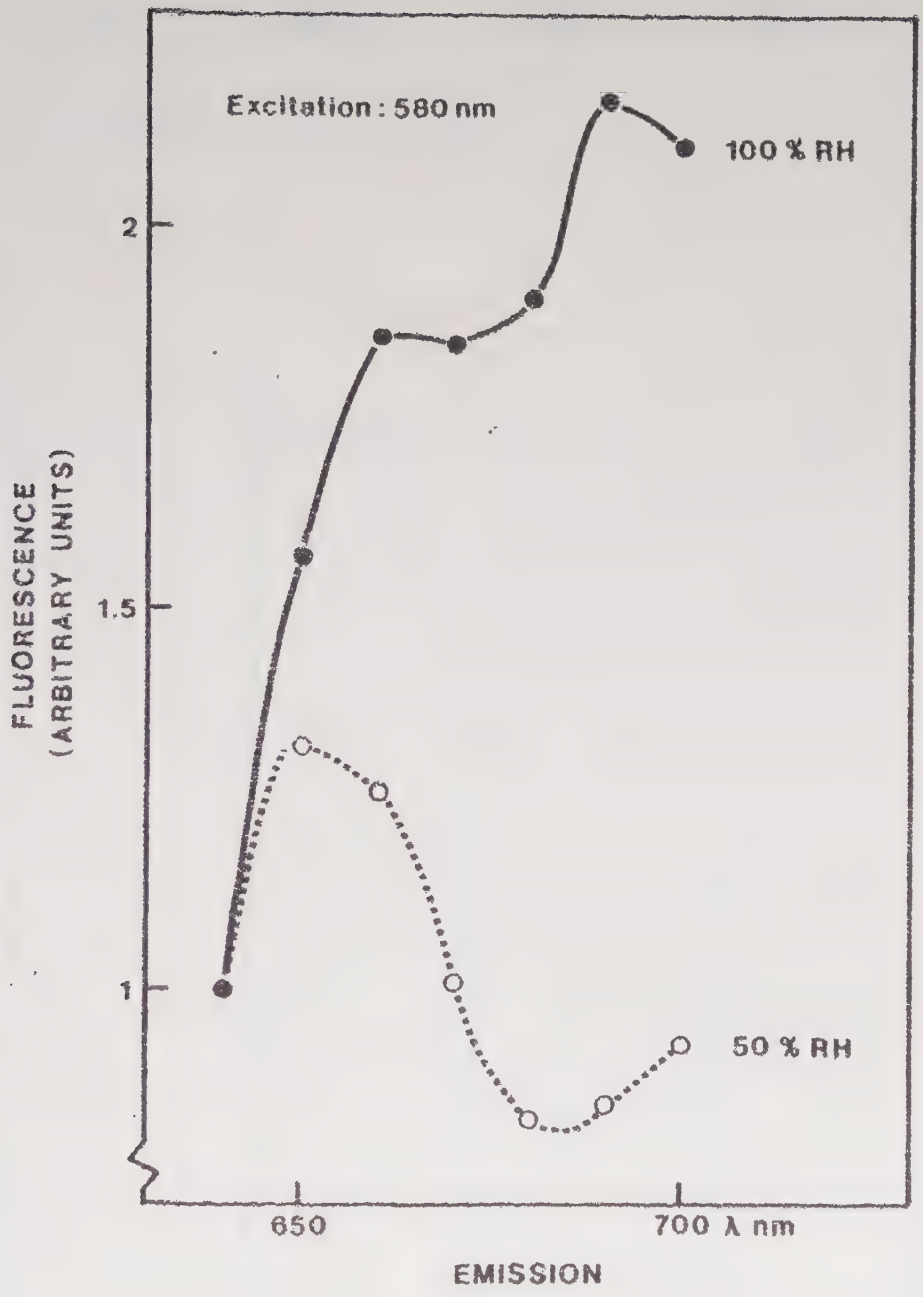
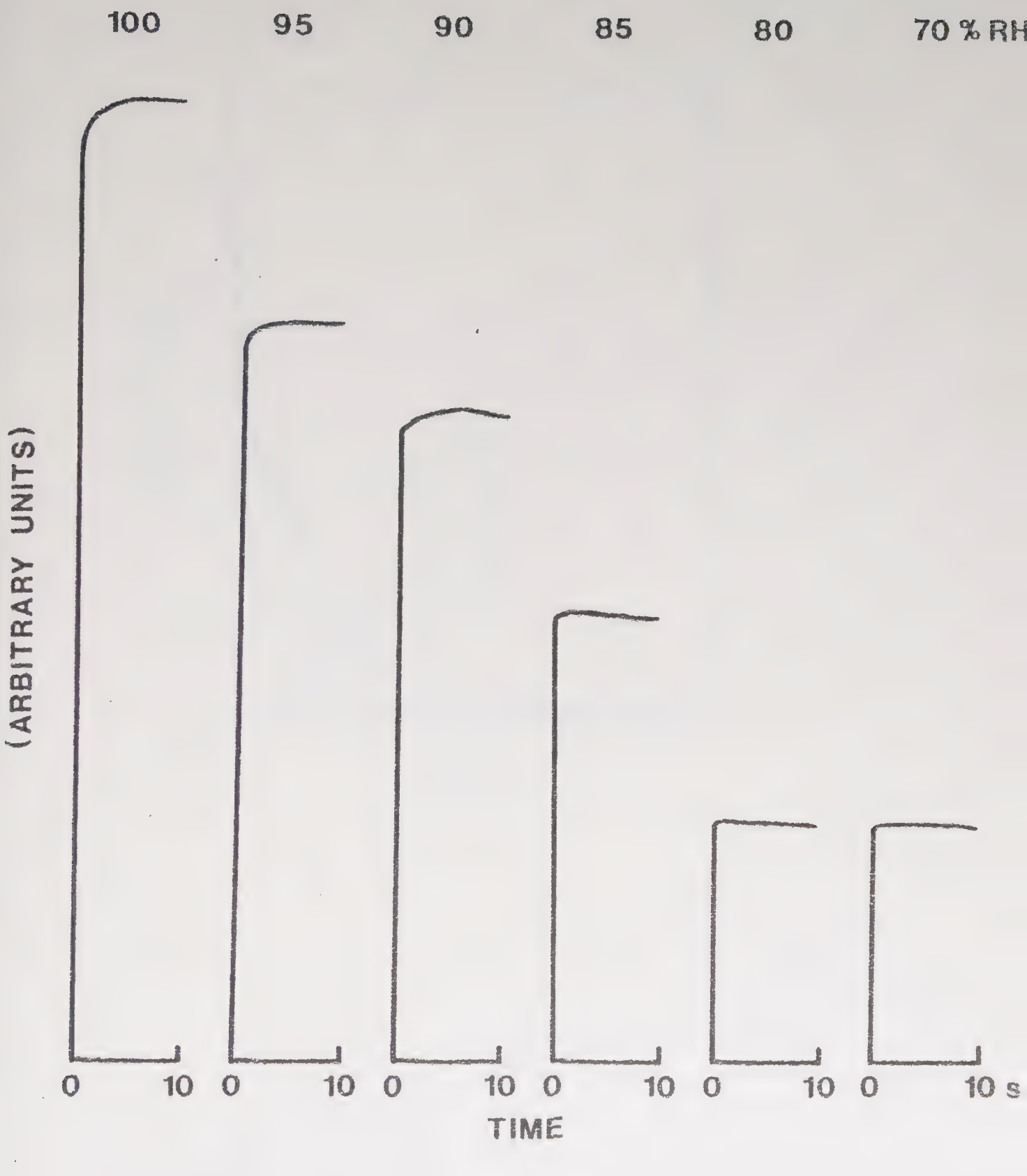
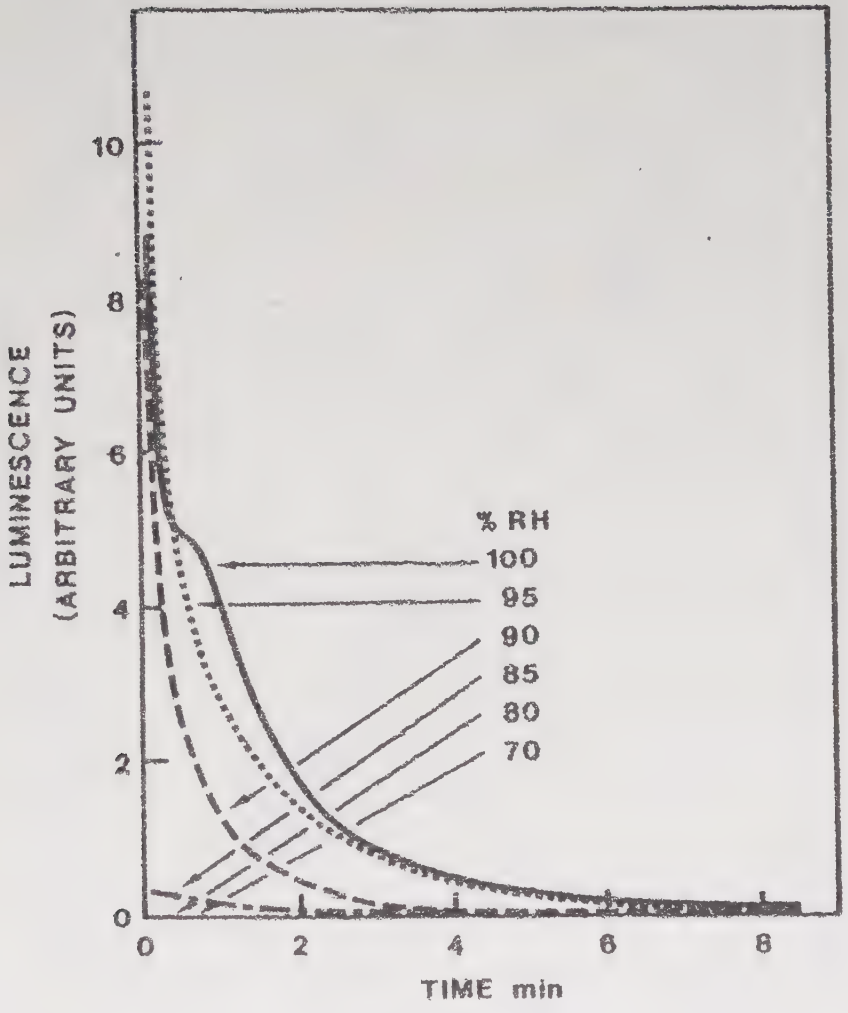
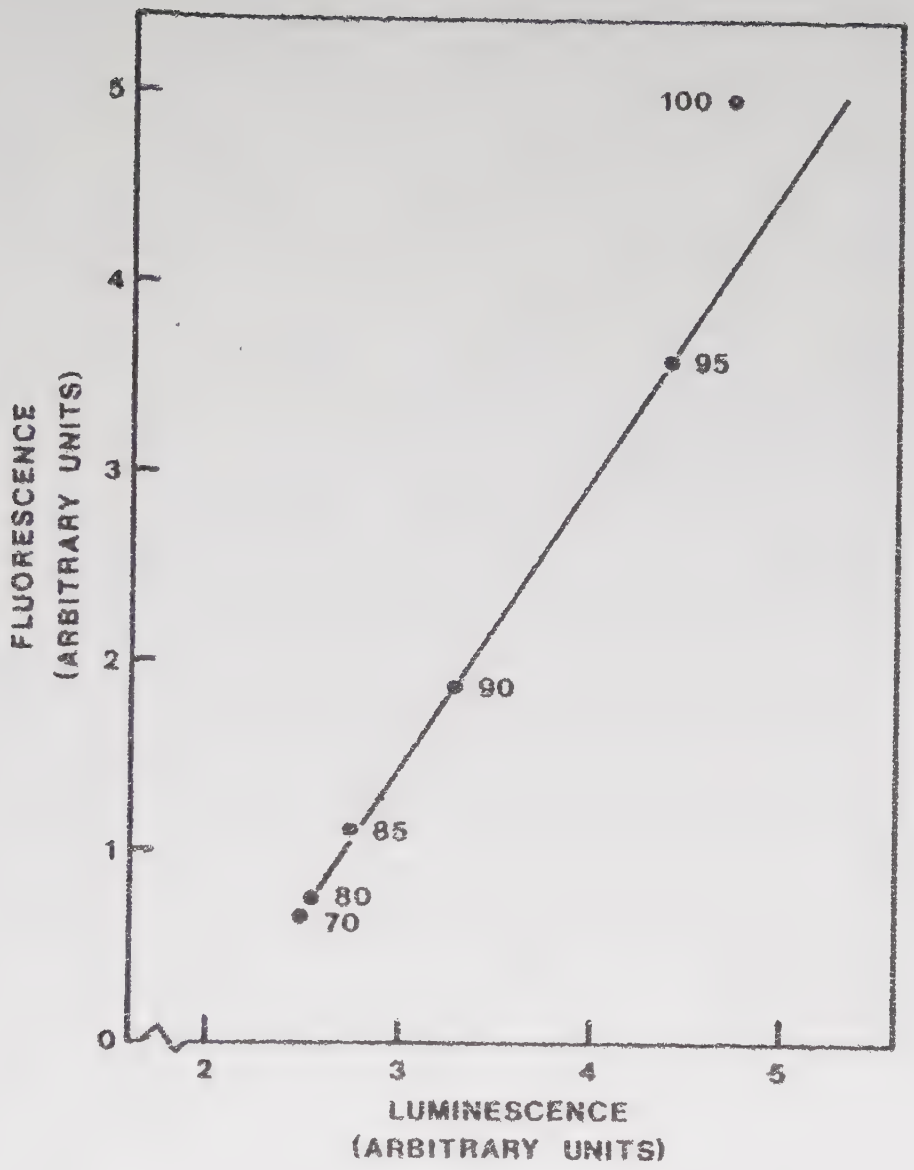

Excitation : 580 nm
100 % RH
50 % RH
2
1.5
1
FLUORESCENCE
(ARBITRARY UNITS)
650
700 λ nm
EMISSION

Fig. 7


100
95
90
85
80
70 % RH
(ARBITRARY UNITS)
0
10
0
10
0
10
0
10
0
10
0
10 s
TIME


LUMINESCENCE
(ARBITRARY UNITS)
10
8
6
4
2
0
% RH
100
95
90
85
80
70
0
2
4
6
8
TIME min


FLUORESCENCE
(ARBITRARY UNITS)
0
1
2
3
4
5
2
3
4
5
LUMINESCENCE
(ARBITRARY UNITS)
100
95
90
85
80
70

Preferential Distribution of Excitation Energy Into Photosystem I of Desiccated Samples of the Lichen *Cladonia impexa* and the Isolated Lichen-Alga *Trebouxia pyriformis*.

by

Björn Sigfridsson [1] and Gunnar Öquist
Department of Plant Physiology, University of Umeå,
S-901 87 Umeå, Sweden

1) Permanent address: Department of Plant Physiology, University of Lund, Box 7007, S-220 07 Lund 7, Sweden

Accepted for publication in Physiologia Plantarum

Abstract

The effect of desiccation on distribution of excitation energy between the two photosystems has been studied in the lichen Cladonia impexa Harm.; in the green alga Trebouxia pyriformis Archibald, isolated from Cladonia squamosa; and in the non-lichen green alga Scenedesmus obliquus, strain D_3. The method used was to compare the low temperature fluorescence emission of samples equilibrated with air with different humidity prior to freezing in liquid nitrogen.

Desiccation of Cladonia and Trebouxia caused a pronounced increase of the height of the far red fluorescence emission band, F 715, over the short wave bands, F 685 and F 697; the ratio between the two short wave bands remained essentially constant. Upon rewetting, these species regained normal fluorescence emission properties, indicating that they are desiccation tolerant. Scenedesmus, which was used as a desiccation intolerant species, also showed an increase of the far red fluorescence band over the two short wave bands upon desiccation, but the original fluorescence spectrum was not restored upon rewetting.

These results are interpreted as showing that desiccation of tolerant species such as Cladonia and Trebouxia causes a preferential energy distribution into photosystem I. We tentatively believe that desiccation induces conformational changes within the chloroplast thylakoids, thereby controlling distribution of energy between the two photosystems. Furthermore, this change in energy distribution may be of ecological significance as the mechanism by which desiccated lichens or algae avoid photodynamic destruction of the photosynthetic apparatus when photosynthesis is inhibited under dry conditions. By a preferen-

tial distribution of absorbed energy into photosystem I, the organisms avoid the formation of strong, harmful oxidants in photosystem II when photosynthesis is inhibited. It is suggested that β-carotene associated with the far red-absorbing chlorophyll a fraction of the reaction center antenna of photosystem I is the final sink for excess excitation energy in dry, desiccation-tolerant lichens and algae.

Introduction

The photosynthetic capacity of lichens is strongly dependent on air humidity (Bertsch 1966). Photosynthesis is inhibited at low air humidity, but recovers rapidly if the humidity is increased to 100 % RH. In nature lichens are able to recover by absorbing water from fog or early morning dew, even when exposed to the moisture for only short periods of time. This points to an on-off mechanism regulating photosynthetic capacity. Laboratory studies of Cladonia impexa have shown that lowered RH induces a reduced energy transfer from chlorophyll b to PS II, inhibited electron transport between PS II and PS I, impared far-red-induced afterglow, reflecting photophosphorylation, and lowered fluorescence yield (Sigfridsson 1980).

When photosynthesis is inhibited in light-exposed desiccated lichens, chlorophyll will be excited in excess, and some of the chlorophyll molecules may be converted to the metastable triplet state. These molecules have the potential to catalyze photodynamic damages, i.e. photooxidation, of chloroplast membrane components. However, such damage does not seem to occur; dry, light-exposed lichens recover photosynthetic ability shortly after rewetting. According to Krinsky (1971), one way that plants avoid photooxidation is through the protective role of caro-

teroids (including β-carotene), which may quench energy absorbed in excess by chlorophyll through one or more of three proposed mechanisms. Energy migration within and between the antennae of photosynthesis is assumed to be directed towards chlorophyll forms absorbing in far red (Seely 1973). These chlorophyll forms should be the ones most susceptible to photooxidation, as is the case in isolated chloroplasts (Brown and French 1979). However, Öquist et al. (1980) have recently given evidence for a far red-absorbing chlorophyll a -β-carotene association in the chlorophyll a antenna of PS I which is believed to function as a trap for excess excitation energy.

Based on isolation with SDS-PAGE, three major chlorophyll-protein complexes have been obtain so far. These are the reaction center chlorophyll a-protein complexes of PS I and PS II, denoted CP-a_I and CP-a_{II}, and the light harvesting chlorophyll a/b-complex, CP-a/b (Thornber 1975, Bishop and Öquist 1980). Based on these three chlorophyll-protein complexes, Butler and coworkers (Butler 1978) have suggested a tripartite model for the in vivo chlorophyll antennae association. According to their model the low temperature fluorescence emission bands F 685, F 697 and F 715 (about 735 nm in higher plants) originate from the in vivo associations of CP-a/b, CP-a_{II} and CP-a_I respectively. The model also proposes that excitation energy is distributed equally between the two photosystems to obtain an optimal quantum yield for photosynthesis.

Treatment with DCMU as well as desiccation of the lichen Cladonia impexa inhibits electron transport between the two photosystems (Sigfridsson 1980). Although this kind of inhibition normally increases fluorescence yield from PS II, the fluorescence yield in desiccated lichens was lowered as compared with a moist control. Based on this

observation and the suggestion that the far red-absorbing chlorophyll-a-β-carotene association in the antenna of PS I is a trap for excess excitation energy (Öquist et al. 1980), we have formulated the hypothesis that desiccated lichens avoid photooxidation by linking excitation energy from PS II into the weakly fluorescent antenna of PS I, where excess excitation energy ultimately would be quenched by β-carotene. As the low temperature fluorescence bands F 685, F 697 and F 715 originate from the discrete antennae of the tripartite model and as the relative heights of these fluorescence bands are affected by energy distribution between the antennae, we have used low temperature fluorescence emission spectroscopy to test if desiccation of the lichen Cladonia impexa and the isolated lichen alga Trebouxia pyriformis causes a preferential transfer of excitation energy into PS I. To get a comparison between the energy distribution of Trebouxia and that of other algae, low temperature measurements have been made on Scenedesmus.

Materials and Methods

Plant material

Cladonia impexa Harm. was collected at Röstånga, near Lund, Sweden. The lichen was stored dry in the dark at room temperature and was used within a month after collection. No negative influence of this storage has been observed (Sigfridsson 1980). Before use, the lichen was first activated by being placed in 100 % RH at room temperature

---

Abbreviations: CP-a_I, photosystem I-chlorophyll a-protein complex; CP-a_{II}, photosystem II-chlorophyll a-protein complex; CP-a/b, chlorophyll a/b-protein complex; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F, fluorescence; HW, half band width; PAGE, polyacrylamide gel electrophoresis; PS, photosystem; RH, relative humidity; SDS, sodium dodecyl sulphate.

and daylight for 1-2 days. It was then placed in airtight plexiglass cuvettes (about 1 liter by volume) in a controlled environmental chamber with conditions as follows: photoperiod 17 h, temperature 25°C in light from metal halogen lamps (Osram HQI-E-400 W/DH 50) and 15°C in dark, irradiance 10 $W \cdot m^{-2}$. The irradiance was measured by a LI-170 Quantum/Radiometer (Lambda Inst. Corp. USA). RH was regulated by proper concentrations of sulfuric acid (Stokes and Robinson 1949) at the bottom of the cuvettes (about 150 ml). Only small pieces (approximately 5×5×3 cm) of the lichen were placed in each cuvette to minimize shortage of carbon dioxide. The acclimatization period was 24 h.

The green alga Trebouxia pyriformis Archibald isolated from Cladonia squamosa and obtained from the algal collection at Austin, Texas, USA, was cultivated under aseptic conditions in a medium containing; 10 mM $MgSO_4$, 12 mM KNO_3, 9 mM KH_2PO_4, 46 µM H_3BO_3, 9 µM $MnCl_2$, 0.7 µM $ZnSO_4$, 0.3 µM $CuSO_4$, 0.1 µM MoO_3, 15 µM $Fe_2(SO_4)_3$, 1 mM Na-citrate, 0.8 mM $CaCl_2$, 10 $g \cdot l^{-1}$ peptone, 20 $g \cdot l^{-1}$ glucose. The algae were grown aseptically at 25°C in Erlenmeyer flasks closed with cotton plugs under continuous white light (2 $W \cdot m^{-2}$; determined with an YSI-Kettering model 65 Radiometer) from fluorescent lights (Philips TL 20 W/33). Before the algae were used, they were washed once in water and resuspended in the above medium, minus peptone and glucose.

The green alga Scenedesmus obliquus, strain D_3 (obtained from N.I. Bishop), was grown heterotrophically as described by Bishop (1971). Continuous light (30 $W \cdot m^{-2}$) was provided by fluorescent lights (Philips TL 20 W/55), and the algae were kept in exponential growth by an automatic dilution system. The suspension was bubbled with air, and the temperature was 27°C.

Low temperature fluorescence emission spectra

The spectra were measured at 77 K with an apparatus and a technique described by Öquist (1974). A computer program was used to correct the spectra for variations in the sensitivity of the photomultiplier and the transmission efficiency of the monochromator at different wavelengths. The emission spectra upon exciting preferentially chlorophyll a (Schott interference filter 433 nm, HW 9 nm; 9 µmol quanta $m^{-2} \cdot s^{-1}$) or chlorophyll b (Schott interference filter 477 nm, HW 10 nm; 20 µmol quanta $m^{-2} \cdot s^{-1}$) were recorded. For the measurements on Cladonia, samples were frozen immediately after removal from the incubation cuvettes, either in light or in darkness. For the measurements on Trebouxia and Scenedesmus the algal suspension was soaked onto filter papers. Spectra were recorded immediately after soaking, after soaked filter papers had been dried at 62 % RH in daylight and room temperature, and after dry algae had been reactivated for 4 h in daylight by spraying the filter paper with distilled water. The amount of algae was kept low enough to avoid reabsorption of the emitted light.

To secure good reproducibility only young samples and as uniform as possible were used (Sigfridsson 1980). Although the low temperature measurements varied somewhat in different samples, they all showed the same trend of response to different RH. This trend will be the base for our conclusions.

Results

The low temperature fluorescence emission spectrum of Cladonia, equilibrated in light in an atmosphere of 100 % RH prior to freezing, showed three characteristic peaks or shoulders at approximately 685, 697 and 715 nm, originating from the light-harvesting CP-a/b complex

and from the reaction center antennae of PS II and PS I (CP-a_{II} and CP-a_{I}), respectively (Figure 1). Upon progressive desiccation of the lichen the far red emission band F 715 increased relative to the short wave bands F 685 and F 697, except at 95 % RH when the reverse relation was observed. At 80 and 85 % RH, F 715 dominated strongly. Concomitantly with the increased relative fluorescence at 715 nm in dried lichens, there was a progressive decrease of total fluorescence yield; lichens at 80 % RH showed about 4 times lower fluorescence yield than controls at 100 % RH as indicated in Figure 1. This is, however, only a rough estimate, since different samples had to be used for each individual experiment.

The curves in Figure 1 were obtained by excitation with 433 nm light, which is absorbed preferentially by chlorophyll a. Essentially the same results were obtained by instead exciting chlorophyll b with 477 nm light (Figure 2). The ratios F 715/ F 685 and F 715/ F 697 were in almost all cases higher when exciting with 433 nm light as compared to excitation with 477 nm light (Figure 3 A,B). Irrespective of excitation light, the most pronounced effect of desiccation on the spectral properties of fluorescence occurred around 90 % RH, as indicated by the strongly increasing F 715/ F 685 and F 715/ F 697 ratios during progressive desiccation. The F 697/ F 685 ratio was essentially unaffected by desiccation (Figure 3 C). Thus, irrespective of whether excitation was directed at preferentially the CP-a/b antenna (477 nm), or the reaction center chlorophyll a antennae of PS I and PS II in addition (433 nm), desiccation of Cladonia resulted in a progressively increased energy transfer to PS I (increasing F 715) over PS II (decreasing F 697); and emission from both the light-harvesting CP-a/b complex (F 685) and PS II (F 697) decreased strongly and in parallel

upon desiccation. Note that desiccation also resulted in a small long wave shift of the far red fluorescence peak of PS I from 713 nm to 717 nm (Figures 1 and 2). This indicates that dehydration had some effect on the *in vivo* organization of the far red-emitting chlorophyll *a* molecules. Throughout this investigation, we have routinely used both 433 and 477 nm light for excitation. As essentially similar results were obtained with the two excitation wavelengths, we present in the following results only for 477 nm excitation.

Desiccation of *Cladonia* in darkness (Figure 4) resulted in similar changes in the fluorescence emission spectrum as those shown in Figures 1 and 2, namely, a distribution of energy into PS I when RH decreased from 100 to 90 %. However, F 697 dominated more over F 715 in the dark-treated samples than in the light treated ones. This means, that energy distribution between the two photosystems, besides being sensitive to RH, is light-dark sensitive.

Low temperature fluorescence emission properties of the isolated lichen alge *Trebouxia pyriformis* responded to desiccation similarly to the lichen *Cladonia* (Figure 5). However, the relative intensities of F 697 and F 715 differed from that of the intact lichen. This was possibly due to different pretreatments prior to freezing in liquid nitrogen. When the alga was rewetted, the original fluorescence spectrum was restored (Figure 5). From the similar fluorescence responses of *Cladonia* and *Trebouxia* to desiccation, we conclude that the mechanism responsible for the changes in energy transfer due to drying/rewetting is linked to the alga, and is not directly influenced by the fungal component, except in so far as the water content of the fungus exerts an indirect control on the mechanism.

The finding that low temperature fluorescence spectral properties of the isolated lichen alga Trebouxia responded to desiccation and rehydration in the same way as the lichen Cladonia raised the question if a non-lichen green alga would respond similarly. The alga Scenedesmus obliquus showed the three characteristic low temperature fluorescence emission bands, although at slightly different positions and ratios than in Trebouxia (Figure 6). Desiccation of Scenedesmus also resulted in a dominating emission at 715 nm, but the other two bands F 685 and F 697 were more pronounced in dry Scenedesmus than in dry Trebouxia or Cladonia. However, rewetting of Scenedesmus did not result in a recovery of the spectrum obtained before drying. Instead, rewetted Scenedesmus had a dominating emission peak at 700 nm and a shoulder at 685 nm, possibly indicating that rewetting induced a block in energy transfer to PS I.

Discussion

The gradual increase of the height of F 715 over F 685 and F 697 during desiccation of the lichen Cladonia shows that absorbed excitation energy is distributed in favour of PS I when the photosynthetic rate is reduced by lower RH (Figures 1,2 and 3). The most pronounced changes in energy distribution occurred at about 90 % RH (Figure 3 A,B), which is parallel to an observed inhibition of photosynthesis in Cladonia by desiccation (Sigfridsson 1980). The strongly dominating F 715 peak in desiccated samples of Cladonia or the isolated lichen alga Trebouxia (Figure 5), is interpreted to show that essentially all excitation energy is distributed to PS I in dry samples. The about 4-fold decrease in total fluorescence yield due to desiccation is also consistent with a preferential energy distribution into PS I, which

exhibits a much lower fluorescence yield than PS II, particularly at room temperature, (Goedheer 1972). Isolated CP-a_I also has a lower fluorescence yield than isolated CP-a_{II} and CP-a/b, both at room temperature and at 77 K (Öquist, unpublished data).

Although desiccation of Cladonia affected the fluorescence emission spectra obtained at both 433 and 477 nm excitation similarly (Figures 1 and 2), the ratios F 715 / F 697 and F 715 / F 685 were always lower (Figure 3) when excitation was with 477 nm light (absorbed preferentially by chlorophyll b) than with 433 nm light (absorbed preferentially by chlorophyll a). This is most likely due to a closer energy coupling between the light-harvesting CP-a/b complex and CP-a_{II} than between CP-a/b and CP-a_I. This causes energy absorbed by chlorophyll b to reach CP-a_I via CP-a_{II}, and not directly as when absorbed by chlorophyll a. The almost constant F 697/F 685 ratio during desiccation of Cladonia implies that energy coupling between CP-a/b and CP-a_{II} remained unchanged, although desiccation caused more absorbed excitation energy to be transferred to PS I. CP-a/b and CP-a_{II} are both functionally (Butler 1978) and physically (Arntzen 1978) associated.

The different effects of desiccation and rehydration on the low temperature fluorescence properties of the lichen alga Trebouxia and the non-lichen alga Scenedesmus (Figures 5 and 6) are believed to be due to the fact that Trebouxia, like the lichen Cladonia, is a desiccation-tolerant species, whereas Scenedesmus has not developed this ability. In studies of the photosynthetic mechanisms of algae living in the upper littoral zone a distinction has been made between desiccation-tolerant and non-tolerant species. Fork and Hiyama (1973) have shown that desiccation of the red alga Porphyra perforata caused

an inhibition of PS II activity, whereas PS I activity is still partly present. After rewetting, PS II activity was regained. In the species Porphyra tenera which is sensitive to drying, PS II activity was not regained after rewetting. The importance for survival of regaining PS II activity after desiccation has also been shown by Wiltens et al. (1978). In non-tolerant algae recovery was possible only if desiccation had not caused an interrupted electron transport between PS II and PS I. After such a block, desiccation was followed by tissue disintegration and death. Tolerant species could stand desiccation beyond that causing an inhibited electron transport between the two photosystems, as is also the case for Cladonia (Sigfridsson 1980).

According to Wiltens et al. (1978) the presence of dehydration tolerance in some species but not in others is due to the higher tolerance of the former species to increased solute concentration and osmotic potential along with conformational changes in proteins and other membrane components. We tentatively believe that conformational changes which occur in the chloroplast thylakoids during desiccation, cause inhibition of photosynthesis and massive energy distribution into PS I; the events occur in parallel and are most pronounced around 90 % RH upon desiccation (Figure 3; Sigfridsson 1980). The earlier observed inhibition of PS II in desiccated algae may well result from a preferential energy distribution into PS I.

One may speculate on the ecological significance of directing absorbed excitation energy into PS I in desiccated lichens. There are reasons to believe that PS I can handle excitation energy absorbed in excess better than PS II and better avoid photodynamic damages. It is known that persistent PS II activity, when over-all electron tran-

sport is stopped, causes the formation of strong oxidants which induce bleaching of carotenoids and chlorophylls (Fujita and Suzuki 1973, Suzuki and Fujita 1977). This is obviously avoided in dry Cladonia which shows no energy transfer into the reaction center antenna of PS II. In PS I excitation energy can be trapped even by oxidized P 700 (Butler 1978), and there is strong evidence that β-carotene associated with the far red-absorbing chlorophyll a forms of PS I provides a final trap for excess excitation energy (Junge et al. 1977, Öquist et al. 1980). The content of β-carotene in CP-a_I isolated from Trebouxia was the same as in CP-a_I isolated from Pisum sativum or Scenedesmus (Öquist et al. 1980, and unpublished data). We therefore believe that the massive energy transfer into PS I in dry Cladonia is a way of avoiding the formation of strong, harmful oxidants in PS II, and that PS I has the necessary photoprotective mechanism, tentatively believed to be β-carotene molecules associated with the reaction center antenna.

A regulatory mechanism balances the distribution of absorbed excitation energy between the two photosystems so that an optimal quantum yield for photosynthesis is achieved (Myers 1971). Principally, two mechanisms have been proposed: 1) regulation of the initial quanta distribution into PS II and PS I, or 2) spill-over of excitation energy from PS II to PS I when PS II receives excitation energy in excess. The relative importance of these two possibilities is still under debate, but Butler and coworkers have included both in their tripartite model for explaining light absorption and utilization (Butler 1978). Light exposure of dark-adapted plants gives a preferential excitation of PS II (State -1), whereas a few minutes illumination causes conformational changes in the chloroplast thylakoids so the two photosystems become equally excited (State -2; Govindjee and Papageorgiou

1971). The finding that the ratio F 715/F 697 was lower in dark-adapted than in light-adapted Cladonia is consistent with a light-regulated energy distribution by conformational changes as in algae (Murata 1968). Although we cannot from our experiments deduce if desiccation affects energy distribution by one or the other mechanism, we can hypothesize that conformational changes similar to those which regulate the State-1 and State -2 transition are also involved in a desiccation-regulated energy distribution. According to such a view, the desiccated chloroplast thylakoids of Cladonia and Trebouxia should be characterized by an extreme State -2 conformation, i.e. low fluorescence yield both at room temperature (Sigfridsson 1980) and at low temperature (Figure 1), and a massive energy distribution into PS I. These conformational changes would thus provide the mechanism by which photosynthesis of lichens is turned off and on by desiccation and rehydration, respectively.

Figure legends

Figure 1. Low temperature (77 K) fluorescence emission spectra of Cladonia impexa equilibrated in light with air at different relative humidity (RH) prior to freezing, as indicated in the figure. The spectra have been normalized to the same area. In order to achieve this, the spectrum of Cladonia desiccated at 80 % RH was multiplied approximately 4 times as compared with the moist lichen samples. Excitation light, 433 nm.

Figure 2. Low temperature (77 K) fluorescence emission spectra of Cladonia impexa with excitation light 477 nm. Otherwise as for Figure 1.

Figure 3. Low temperature fluorescence peak ratios (A) F 715/F 685, (B) F 715/F 697 and (C) F 697/ F 685 of Cladonia impexa equilibrated with air at different relative humidity (RH) prior to freezing. The calculated ratios are based on data shown in Figures 1 (433 nm light) and 2 (477 nm light).

Figure 4. Low temperature (77 K) fluorescence emission spectra of Cladonia impexa equilibrated, in light (L) or in darkness (D), with air at 90 or 100 % relative humidity (RH) prior to freezing. The spectra have been normalized to the same area. Excitation light 477 nm.

Figure 5. Low temperature (77 K) fluorescence emission spectra of the isolated lichen green alga Trebouxia pyriformis when wet (W), dried at 62 % relative humidity (RH) or rewetted (RW) by distilled water prior to freezing. The pretreatment was performed in light. The spectra have been normalized to the same area. Excitation light 477 nm.

Figure 6. Low temperature (77 K) fluorescence emission spectra of the non-lichen green alga Scenedesmus obliquus when wet (W), dried at 62 % relative humidity (RH) or rewetted (RW) by distilled water prior to freezing. The pretreatment was performed in light. The spectra have been normalized to the same area. Excitation light 477 nm.

References

Arntzen, C.J. 1978. Dynamic structural features of chloroplast lamellae. - In Current Topics in Bioenergetics (D.R. Sanadi and L.P. Vernon, eds.) Vol. 8, pp. 111-160. Academic Press, New York, San Francisco, London.

Bertsch, A. 1966. Ueber den CO_2-Gaswechsel einiger Flechten nach Wasserdampfaufnahme. - Planta 68:157-166.

Bishop, N.I. 1971. Preparation and properties of mutants; Scenedesmus. - In Methods in Enzymology (S.P. Colowick and N.O. Kaplan, eds.) Vol. 23 A, pp. 130-143. Academic Press, New York.

Bishop, N.I. & Öquist, G. 1980. Correlation of the photosystem I and II reaction center chlorophyll-protein complexes, CP-a_I and CP-a_{II}, with photosystem activity and low temperature fluorescence emission properties in mutants of Scenedesmus. - Physiol. Plant. In print.

Brown, J.S. & French, C.S. 1959. Absorption spectra and relative photostability of the different forms of chlorophyll in Chlorella. - Plant Physiol. 34:305-309.

Butler, W.L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. - Annu Rev. Plant Physiol. 29:343-378.

Fork, D.C. & Hiyama, T. 1973. The photochemical reactions of photosynthesis in an alga exposed to extreme conditions. - Carnegie Inst. Yearbook 72:184-188.

Fujita, Y. & Suzuki, R. 1973. Studies on the Hill reaction of membrane fragments of blue-green algae IV. Carotenoid photobleaching induced by photosystem II action. - Plant Cell Physiol. 14:261-273.

Goedheer, J.C. 1972. Fluorescence in relation to photosynthesis. - Annu. Rev. Plant Physiol. 23:87-112.

Govindjee & Papageorgiou, G. 1971. Chlorophyll fluorescence and photosynthesis: Fluorescence transients. - In Photophysiology (A.C. Giese, ed.) Vol. 6, pp. 1-46. Academic Press, New York, London.

Junge, W., Schaffernicht, H. & Nelson, N. 1977. On the mutual orientation of pigments in photosystem I particles from green plants. - Biochim. Biophys. Acta 462:73-85.

Krinsky, N.I. 1971. Function. - In Carotenoids (O. Isler, ed.) pp. 669-716. Birkhäuser Verlag, Basel and Stuttgart.

Murata, N. 1968. Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll a fluorescence in Porphyridium cruentum. - Biochim. Biophys. Acta 172:242-251.

Myers, J. 1971. Enhancement studies in photosynthesis. - Annu. Rev. Plant Physiol. 22:289-312.

Öquist, G. 1974. Iron deficiency in the blue-green alga Anacystis nidulans: Fluorescence and absorption spectra recorded at 77 K. - Physiol. Plant. 31:55-58.

Öquist, G., Samuelsson, G. & Bishop, N.I. 1980. On the role of β-carotene in the reaction center chlorophyll a antenna of photosystem I. - Physiol. Plant. In press.

Seely, G.R. 1973. Effects of spectral variety and molecular orientation on energy trapping in the photosynthetic unit: A model calculation. - J. Theor. Biol. 40:173-187.

Sigfridsson, B. 1980. Some effects of humidity on the light reaction of photosynthesis in the lichens Cladonia impexa and Collema flaccidium. - Physiol. Plant.

Stokes, R.H. & Robinson, R.A. 1949. Standard solutions for humidity control at 25°C. - Industr. Engng. Chem. 41, p. 2013.

Suzuki, R. & Fujita, Y. 1977. Carotenoid photobleaching induced by the action of photosynthetic reaction center II: DCMU-sensitivity. - Plant Cell Physiol. 18:625-631.

Thornber, J.P. 1975. Chlorophyll-proteins: light-harvesting and reaction center components of plants. - Annu. Rev. Plant Physiol. 26:127-158.

Wiltens, J., Schreiber, U. & Vidaver, W. 1978. Chlorophyll fluorescence induction: an indicator of photosynthetic activity in marine algae undergoing desiccation. - Can. J. Bot. 56:2787-2794.

Fig. 1

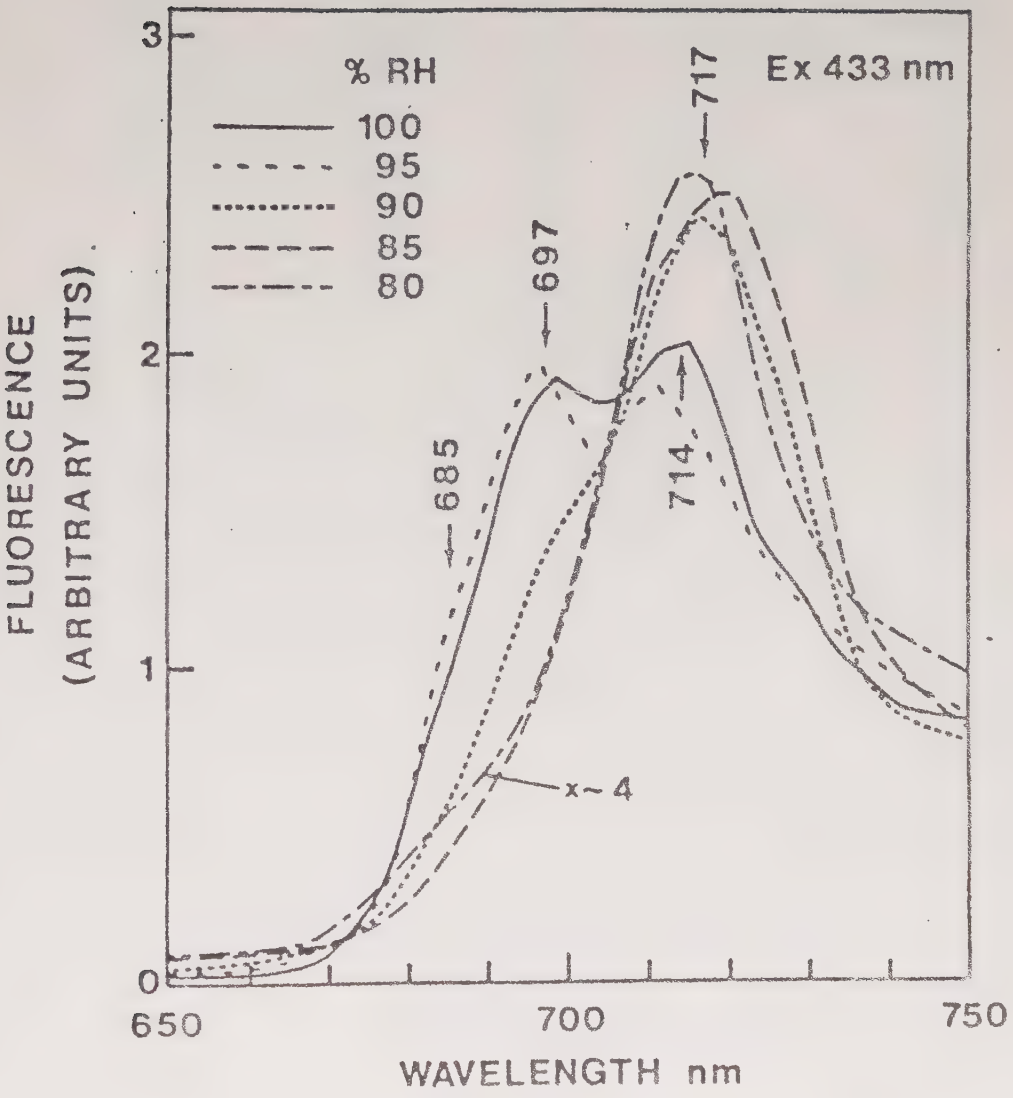

% RH
100
95
90
85
80
Ex 433 nm
717
697
685
714
x~4
FLUORESCENCE
(ARBITRARY UNITS)
0
1
2
3
650
700
750
WAVELENGTH nm

Fig. 2

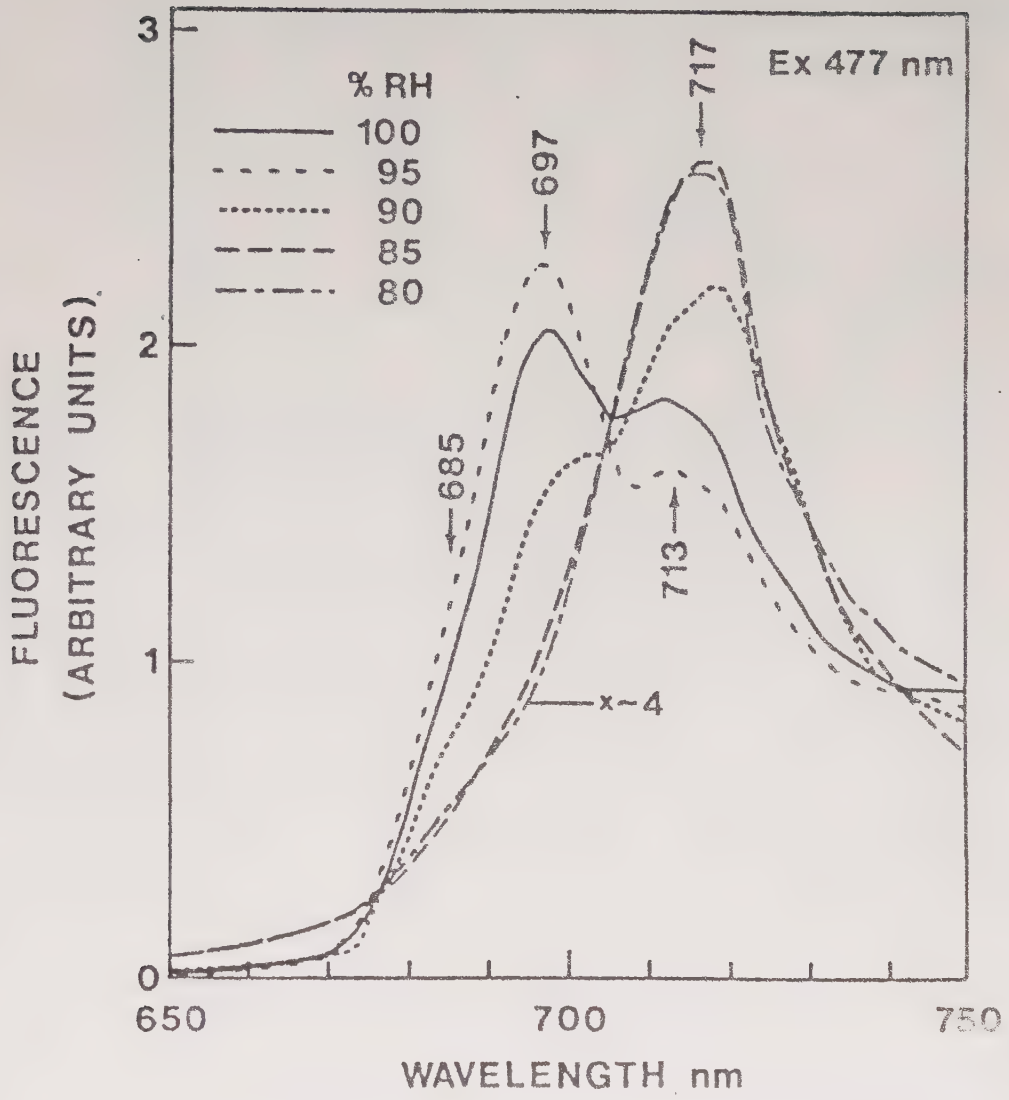
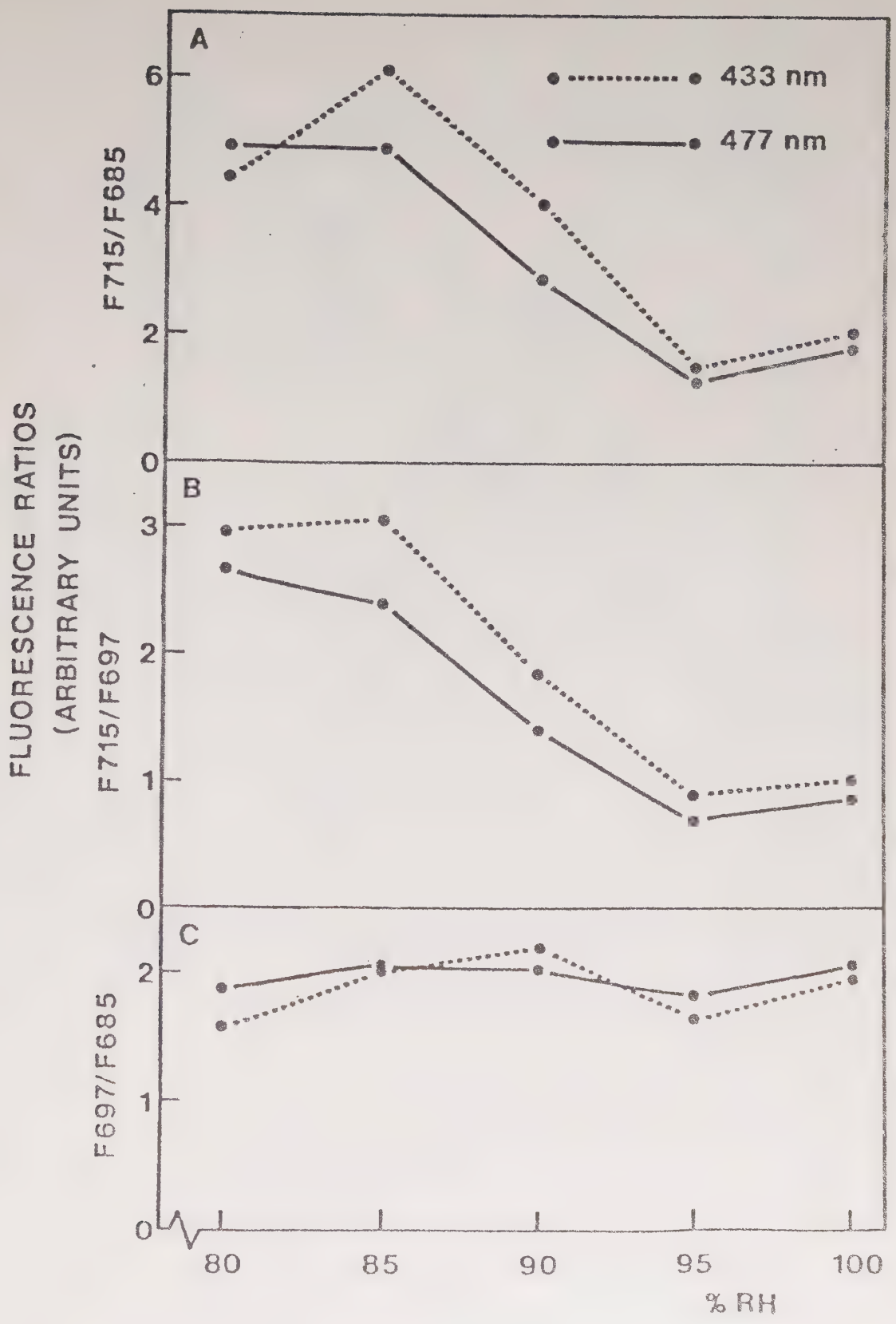
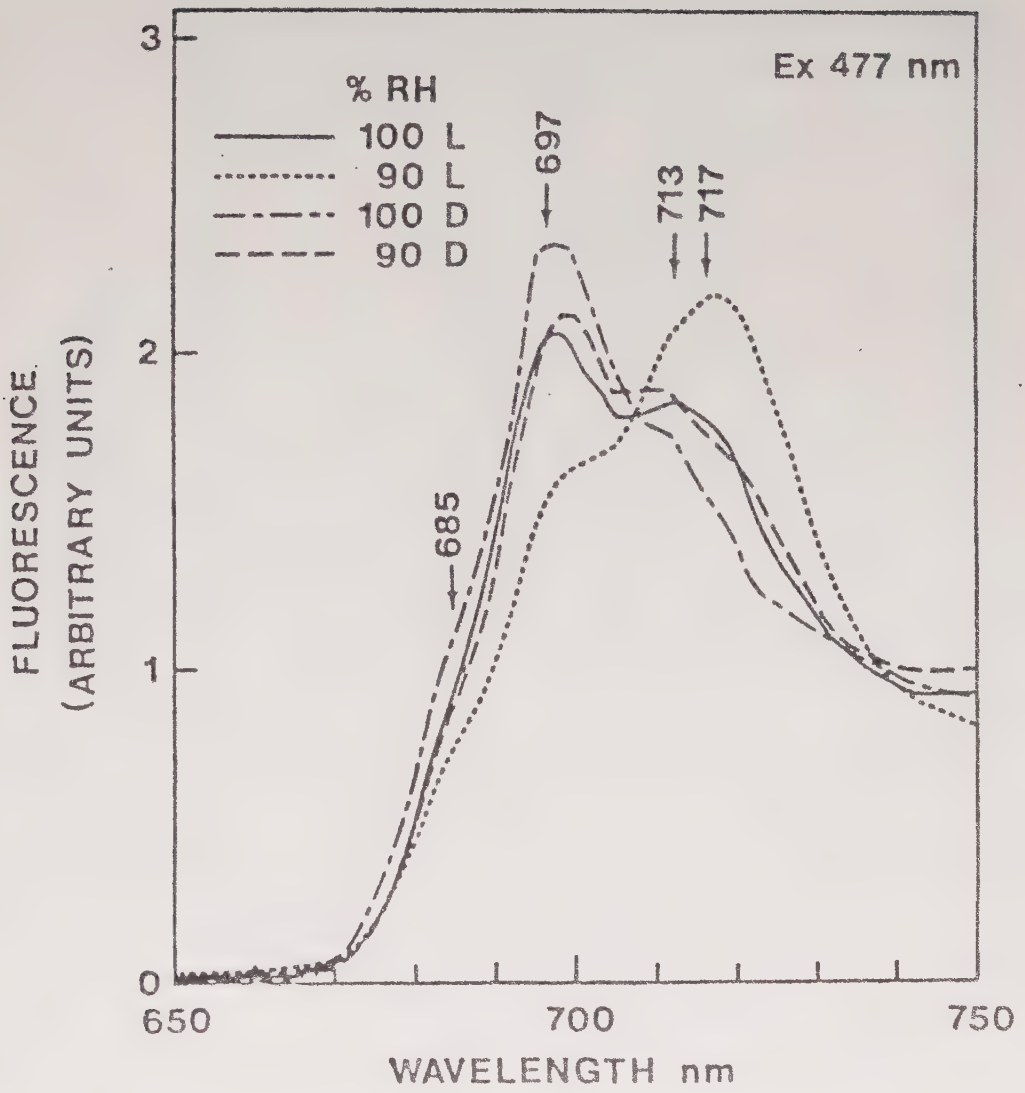
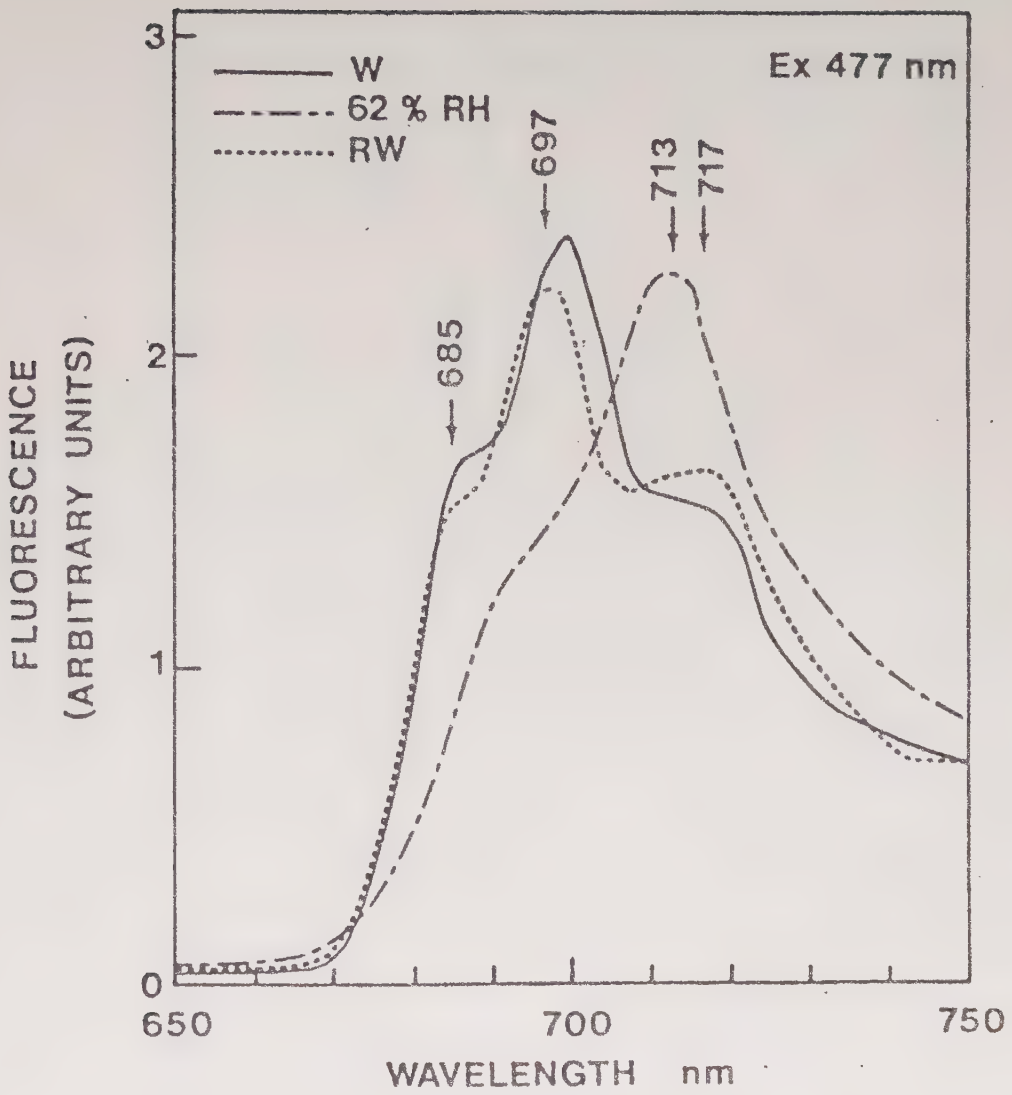
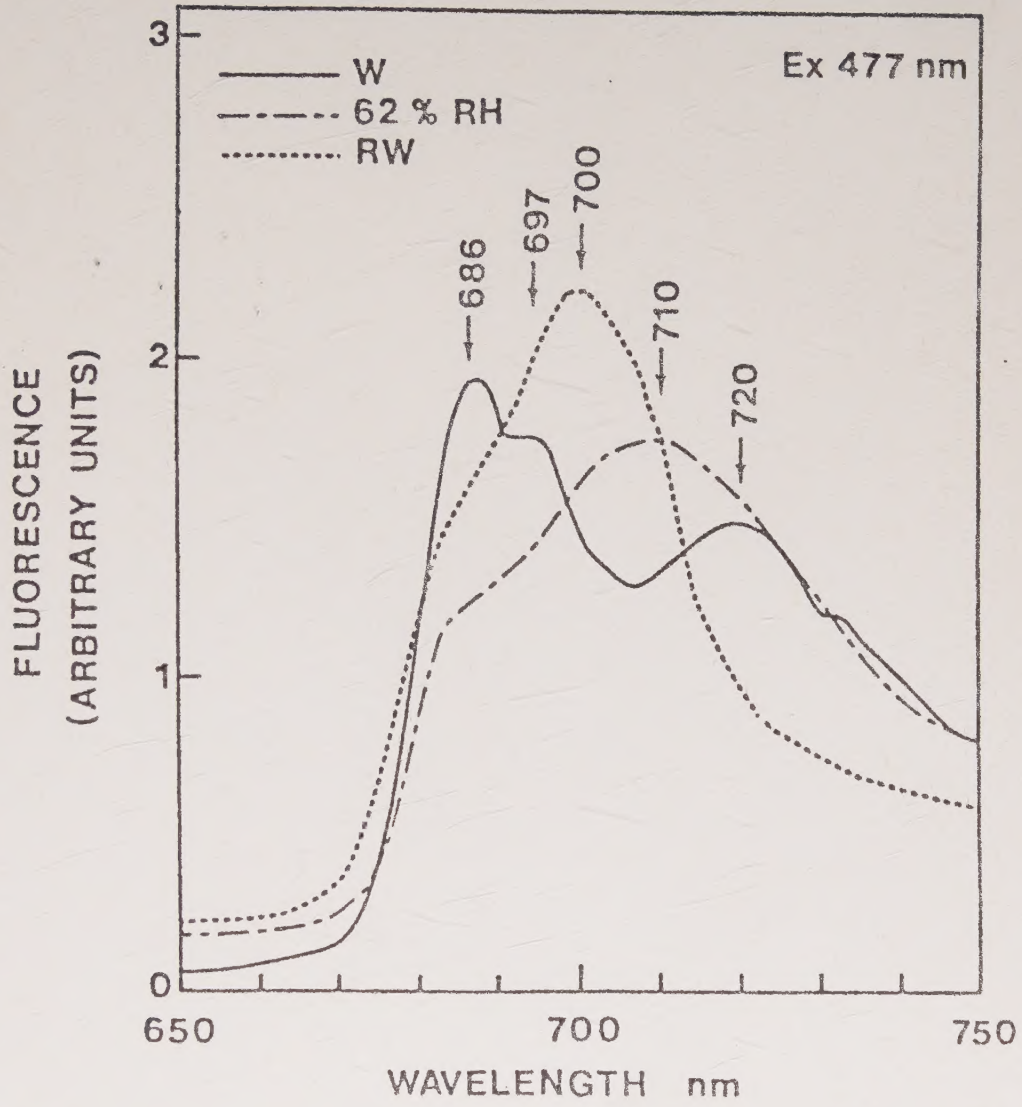

% RH
100
95
90
85
80
Ex 477 nm
717
697
685
713
x~4
FLUORESCENCE
(ARBITRARY UNITS)
3
2
1
0
650
700
750
WAVELENGTH nm

Fig. 3


A
B
C
433 nm
477 nm
F715/F685
F715/F697
F697/F685
FLUORESCENCE RATIOS
(ARBITRARY UNITS)
0
1
2
3
4
6
80
85
90
95
100
% RH


Ex 477 nm
% RH
100 L
90 L
100 D
90 D
697
713
717
685
FLUORESCENCE
(ARBITRARY UNITS)
0
1
2
3
650
700
750
WAVELENGTH nm


W
62 % RH
RW
Ex 477 nm
685
697
713
717
FLUORESCENCE
(ARBITRARY UNITS)
0
1
2
3
650
700
750
WAVELENGTH nm


W
62 % RH
RW
Ex 477 nm
686
697
700
710
720
FLUORESCENCE
(ARBITRARY UNITS)
0
1
2
3
650
700
750
WAVELENGTH nm